EFFICACY OF SOME PLANT EXTRACTS IN CONTROLLING LEAF BLIGHT OF SUNFLOWER

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EFFICACY OF SOME PLANT EXTRACTS IN CONTROLLING LEAF BLIGHT OF SUNFLOWER

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*This is to certify that thesis entitled, "***EFFICACY OF SOME PLANT EXTRACTS IN CONTROLLING LEAF BLIGHT OF SUNFLOWER***" submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, 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* **MD. ALI HAIDAR**, *Registration No.* **19-10044** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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Dedicated
to
My Beloved Parents and Supervisor

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The author

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ABSTRACT

The experiment was conducted in Central MS laboratory and Central Farm of Sher-e-Bangla Agricultural University, Dhaka-1207 during November 2019 to May 2020 to evaluate the efficacy of some plant extracts in controlling leaf blight of sunflower variety BARI Surjamukhi-2. The causal organism isolated from infected leaf of sunflower and identified as *Alternaria alternata* by tissue planting method. The field experiment was laid out in randomized complete block design (RCBD) with three replications. The treatments consisted of plots sprayed with T_1=Control, T_2=Neem leaf extracts, T_3=Garlic bulb extracts, T_4=Mint leaf extracts, T_5=Allamanda leaf extracts, T_6=Papaya leaf extracts and T_7=Mahogany leaf extracts. Concentration of all plant extracts was 1:3 w/v. Data were collected on disease incidence, percent disease index (PDI), plant height, number of leaves per plant, stem girth, head diameter, number of seeds per head, 1000 seeds weight and yield. The lowest disease incidence (43.32%) with maximum reduction (45.64%) of PDI over control was recorded in garlic bulb extracts (T_3) at 75 DAS. Garlic bulb extracts (T_3) treated plots gave better response in all growth, yield and yield contributing parameters viz., tallest plant (132.4 cm), maximum number of leaves (28) per plant, thickest plant (5.47 cm), maximum head diameter (17.78 cm), highest number of seeds (523) per head, 1000 seeds weight (57.46 g) and yield (778.87 g/plot). The maximum yield increased (63.39%) over control was also recorded in garlic bulb extracts (T_3) followed by papaya leaf extracts (T_6) and neem leaf extracts (T_2). In this study it was found that spray with garlic bulb extracts (T_3) showed most effective in controlling *Alternaria* leaf blight incidence, severity with increasing yield of sunflower.

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ABBREVIATIONS AND ACRONYMS

SAU	=	Sher-e-Bangla Agricultural University
BBS	=	Bangladesh Bureau of Statistics
AEZ	=	Agro-Ecological Zone
USDA	=	United States Department of Agriculture
WHO	=	World Health Organization
FAO	=	Food and Agricultural Organization
MS	=	Master of Science
RCBD	=	Randomized Complete Block Design
et al.,	=	And others
viz.,	=	Namely
J.	=	Journal
e.g.	=	exempli gratia (L), for example
etc.	=	Etcetera
i.e.	=	id est (L), that is
%	=	Percentage
°C	=	Degree Celsius
mg	=	Miligram
L	=	Litre
Mg	=	Microgram
cm	=	Centimeter
m^2	=	Meter square
ml	=	Milliliter
g	=	Gram (s)
Kg	=	Kilogram (s)
LSD	=	Least Significant Difference
No.	=	Number
CV	=	Coefficient of Variation
DAS	=	Days After Sowing
PDA	=	Potato Dextrose Agar

CHAPTER I

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an important oil seed crop and ranked fourth in worldwide among the vegetables oil seed production. The genus *Helianthus* is named from the Greek word helios meaning sun and anthos meaning flower (Kindscher, 1987). The family of sunflower is Asteraceae (Compositae) and the origin is southern USA and Mexico (Heiser, 1951). It is extensively grown in Ukraine, Russia, Argentina, Romania, China, Turkey, Bulgaria, Hungary, France, USA, Spain, and India.

In Bangladesh, Sunflower is being cultivated as an oil seed crop since 1975 (Islam *et al*., 2004). It can be a profitable first crop in double or multiple cropping system (Sheaffer *et al*., 1977). It can also be cultivated as an irrigated or rain fed crop in areas where the other oil seed crop like groundnut cannot be grown. Now-a-days special emphasis has given to extend sunflower production in saline and char regions in the country. In 2018-19, Bangladesh produced about 1975 metric tons' sunflower from 3187 acres' land (BBS, 2020).

Sunflower oil contributes about 13% of the world edible oil production with high value (Gabagambi *et al*., 2010). Its seed is highly nutritious containing about 20% protein and 40 to 50% vegetable oil associated with a very high calorific value. The oil is considered to be of high quality due to its non-cholesterol properties and has been recommended for the patient having heart problem. It contains 60 to 73% linoleic acid, with sufficient amount of calcium, iron and vitamins like A, B, E and K (Gosal *et al*., 1988). The oil cake is rich in high quality protein (40-44%) and used as cattle and poultry feed. As a source of high-quality edible oil, sunflower oil is used as cooking oil in different food preparations (Anonymous, 2005; Joksimovic *et al.*, 2006). It can also be used as manure (Agy *et al.*, 2013). *H. annuus* is a plant with not only food and energy

values, but also with phytoremediation potential (Mukhtar *et al*., 2010; Seth *et al*., 2011).

Sunflower suffers from many diseases caused by fungi, bacteria and viruses. It is the known host of more than 30 pathogens mostly fungi which under certain climatic condition may impair the normal physiology of the plant so that yield and oil quality are reduced significantly (Gulya *et al*., 1994). Some of the most important fungal diseases of sunflower are *Alternaria* leaf blight (*Alternaria* sp.), Rust (*Puccinia helianthi*), Powdery mildew (*Erysiphe cichoracearum*), Downy mildew (*Plasmopara halstedii*), Root rot (*Macrophomina phaseolina*), Collar rot (*Sclerotium rolfsii*), Head rot (*Rhizopus* sp.), Verticillium wilt (*Verticillium dahliae*) and Leaf spot (*Helminthosporium helianthi*).

Among these diseases, *Alternaria* leaf blight is one of the major diseases of sunflower (Prathibha *et al*., 2008; Prasad *et al*., 2020) that can reduce the seed yield by 28% to 81% and oil content by 19% to 34% (Reddy and Gupta, 1977; Balasubrahmanyam and Kolte, 1980).

Nine species of *Alternaria* have been reported on sunflower, including *A. alternata* (Fries.) Kiessler, *A. helianthicola* Rao and Rajagopalan, *A. helianthinficiens* Simmons, *A. leucanthemi* Nelen (syn. *A. chrysanthemi* Simmons and Grosier), *A. longissima* Deighton and MacGarvey, *A. protenta* Simmons, *A. tenuissima* (Fries) Wiltshire and *A. zinniae* Ellis (Lapagodi and Thanassoulopoulos, 1998).

The pathogen *Alternaria* sp. over winters as mycelium or conidia in plant debris, soil, infected tubers or on other host plants of the same family. The conidiophores of *A. alternata* were simple, branched, straight or flexuous, olivaceous or golden brown. Septate conidia were muriform in shape measured 35.21 µm long and 13.47 µm width (Mallikarjun, 1996). The pathogenic fungi can invade the discs, stems, and leaves of sunflower, causing round brown lesions, leaf drop, and even death of the plants. The disease can also result in premature defoliation under conditions of warm temperature and high relative humidity (Prathuangwong *et al*., 1991).

The disease is controlled primarily through the use of cultural practices such as, crop rotation, tillage, removal and burning of infected plant debris and eradication of weed hosts reducing the inocula level for subsequent plantings, using resistant cultivars and foliar fungicides are considered as substantial options. No doubt, stable resistant varieties are the most feasible, effective, economical, efficient, practical and environmentally safe method to manage this disease. Until now, there is no resistant variety or hybrid available against this disease. The most common and effective method for the control of leaf blight of sunflower is the application of foliar fungicides, but the fungicides treatment pollute environment, increase cost of plant protection, effect health vulnerability in humans and when these harmful chemicals enter into the food chain become hazardous to all living entities. Botanical derivatives are environmentally safe, economically cheap and may be used as an alternative to commercial fungicides for controlling pathogenic fungi.

There are hundreds of plant product that have a long history of antimicrobial properties against various plant pathogens. Screening of plant products for these antimicrobial activities is very essential and needs urgent attention in order to know the real value of our national plant genetic resources. The toxic substances obtained from various plant species, manage a number of fungal diseases of crop plants (Raghav, 2003). Several authors (Buckingham, 1993; Maji *et al*., 2005; Patni *et al*., 2005) have reported use of botanicals as biorationals. Few workers (Shekhawat and Prasad, 1971; Mesta *et al*., 2009; Prasad *et al*., 2013; Devi *et al*., 2013 and Rakholia *et al*., 2016) evaluated different botanicals against *Alternaria* leaf blight of sunflower under *in vitro* and *in vivo* conditions. Shekhawat and Prasad (1971) reported that out of nine plant extracts tested, five viz., *Allium cepa, A. sativum, Ocimum sanctum, Mentha piperita* and *Beta vulgaris* showed strong inhibitory action against *Alternaria* sp. Rakholia *et al*. (2016) reported that garlic cloves extract was highly inhibitory to the growth of *Alternaria alternata* causing ripe fruit rot of chilli followed by ardusi leaf extract and gave 58.96 and 33.83 percent inhibition respectively. Prasad *et al*. (2013) found that among the plant extracts, garlic showed disease reduction of 52.0% over

pathogen check followed by neem under field condition. The screening of plants for their biologically active principles is done on the basis of either their chemotaxonomic investigation or ethno-botanical knowledge and literature for *Alternaria* blight disease.

Considering above facts and points this research work was designed to achieve the following specific objectives:

1. To isolate and identify the causal agent of leaf blight of sunflower
2. To evaluate the efficacy of some plant extracts in controlling leaf blight disease of sunflower in field condition.

CHAPTER II

REVIEW OF LITERATURE

Sunflower (*Helianthus annuus* L.) is an important oilseed crop grown all over the world. This crop suffers from many diseases, among which leaf blight is the most serious disease that cause considerable yield loss in many sunflower growing areas. In this chapter, an attempt has been made to review the work done on various aspects like isolation, identification, proving pathogenicity and management of *Alternaria* leaf blight of sunflower by various plant extracts with different headings and sub-headings.

2.1. Importance of sunflower

Sunflower (*Helianthus annus* L.) is well known as a flower as well as a promising oil seed crop in Bangladesh. There is high demand for sunflower because its oil is good for health as it contains low cholesterol. It contains 60-73% linoleic acid with sufficient amount of calcium, iron and vitamins like A, B, E and K. Sunflower seed is source of high quality oil (45-52%) have higher content of polyunsaturated fatty acid. 1 kg of sunflower seeds yields 500 to 600 grams of oil, which is more than that of any other oilseeds. It also contains good quality protein (19 to 25%) in seeds (Gosal *et al*., 1988). Moreover, the crop can also be used in animal feeding (Porto *et al.,* 2008) and in the cosmetics industry (Moraes and Paula, 2013).

It is one of the most widely studied plants for heavy metal phytoremediation (Kara *et al*., 2013). However, it is well known that sunflower is able to contain, degrade or eliminate metals (Chen *et al*., 2012; Ker and Charest, 2010; Lee and Yang, 2010), polycyclic aromatic hydrocarbons (Tejeda-Agredano *et al*., 2013) and polychlorinated biphenyls (Fiebig *et al.,* 1997) from soil or water. Sunflower species are allelopathic in nature; as well as cultivated sunflower has great

allelopathic potential and inhibits weed-seedling growth of velvet leaf, thorn apple, morning glory, wild mustard and other weeds (Macías *et al.*, 1998a).

Farmers are cultivating sunflower as an adaptation practices of climate change in the coastal region of Bangladesh. Due to its larger adaptation capability and higher oil quality, sunflower can be grown almost in all the regions of the world with high seed yield and oil content (Sencar *et al.*, 1991).

2.2. Fungal diseases of sunflower

Sunflower (*Helianthus annuus*) is a known host for over 36 pathogenic organisms, mostly fungi that can cause serious economic losses, depending on the climatic conditions (Gulya *et al*., 1997; Zimmer and Hoes, 1978).

Twenty six different diseases of sunflower have been listed by United States Department of Agriculture (USDA). Among these about fifteen are fungal diseases viz., Gray-mold blight (*Bortytis cinera*), *Cercospora* leaf spot (*Cercospora pachypus*), Powdery mildew (*Erysiphe cichoracearum*), Charcol stem rot (*Macrophomina phaseolina*), Downey mildew (*Plasmopara halstedii*), Rust (*Puccinia helianthi*), Root rot (*Pythim debaryanum*), Violet root rot (*Rhizoctonia crocorum*), Root and stem rot (*Rhizoctonia solani*), Stem rot and wilt (*Sclerotinia sclerotiorum*), Southern blight (*Sclerotium rolfsii*), Leaf spot (*Septoria helianthi*) and Rust (*Uromyces funci*) of sunflower (USDA, 1960).

Richardson (1990) listed 18 seed borne diseases in sunflower. Among these important seed borne fungal pathogens recorded in sunflower were *Alternaria alternata* (Leaf spot and leaf blight), *Alternaria helianthi* (Leaf spot and leaf blight), *Botrytis cinerea* (Grey mold), *Macrophomina phaseolina* (Charcol rot), *Plasmopara halstedii* (Downy mildew), *Puccinia helianthi* (Rust), *Sclerotinia sclerotiorum* (Wilt, White rot, Stem rot) and *Verticillium alboatrum* (Wilt).

In Bangladesh, limited works have been done on the diseases of sunflower. Talukder (1974) listed two diseases, anthracnose (*Colletrotichum* sp.) and stem

rot (*Sclorotium rolfsii*) while Meah (1994) reported *Alternaria* blight or leaf spot (*Alternaria helianthi*) and coller rot (*Sclerotium rolfsii*) in the crop.

Rahman *et al*. (2007) recorded nine species of fungi, in order of prevalence, were- *Alternaria alternata, Botrytis cinerea, Rhizopus stolonifer, Fusarium moniliforme, Fusarium semitectum, Aspergillus niger, Penicillium sp., Aspergillus flavus* and *Curvularia lunata*. Of all these fungi *A. alternata, B. cinerea, F. moniliforme, F. semitectum* and *R. stolonifera* were predominant. All the nine fungi encountered in sunflower seeds appeared to be new records for Bangladesh.

2.3. Etiology

Among the diseases of sunflower, leaf blight incited by *Alternaria* species is reported from all sunflower growing regions across the globe but assumes importance in the tropics and the subtropics (Takano, 1963; Tubaki and Nishihara, 1969; Kolte and Mukhopadhyay, 1973; Shane *et al.*, 1981; Tosi and Zazzerini, 1991).

The disease has been reported from Argentina (Ribeiro *et al*., 1974), North America (Shane *et al*., 1981; Herr and Lipps, 1981), Brazil (Riobeiro *et al*., 1974), Bulgaria (Bchvarova, 1978), Romania (Hulea *et al*., 1973), Yugoslavia (Islam *et al*., 1976; Acimovic, 1977), South Africa (Westhuizen *et al*., 1980), Tanzania (Fivawo, 1987), Australia (Alcorn and Pont, 1972), Japan (Tubaki and Nishihara, 1969) and Pakistan (Bhutta *et al*., 1997).

Alternaria alternata (Fries.) Kiessler recorded on sunflower from Bulgaria, Greece, Argentina, India, Brazil, China, Iran, Thailand, former USSR, Italy and Greece (Lagopodi and Thanassoulopoulos, 1998).

In India, the disease was first recorded and subsequently reported by Narian and Saksena (1973). Kolte and Mukhopadhayay (1973) from Utlar Pradesh and later by Anilkumar *et al*. (1974) from Karnataka. In Karnataka, the disease occurred

in epiphytic from in 1987 with disease incidence as high as 95 to 100 percent (Hiremath *et al*., 1990).

In Bangladesh, Meah (1994) reported that *Alternaria* blight or leaf spot of sunflower is caused by *Alternaria* sp.

2.4. Significance of *Alternaria* leaf blight disease of sunflower

Sunflower leaf blight can reduce the seed yield by 28% to 81% and oil content by 19% to 34% (Balasubrahmanyam and Kolte, 1980; Bai *et al*., 1985).

The *Alternaria* blight is both internally and externally seed borne disease. The economic value of sunflower seeds is greatly influenced by the associated seed borne fungi which may reduce oil quality due to increase of free fatty acids amount in seeds during storage (McGee and Christensen, 1970; Singh and Prasad, 1977). It is one of the most destructive and wide spread disease which causes 57–80% of yield loss under severe epiphytotic conditions (Hiremath *et al*., 1990; Shankergoud *et al*., 2006).

Further, the leaf spot or blight significantly reduces head diameters, seed numbers, 1000-seed weight and oil percentage (Reddy and Gupta, 1977; Balasubrahmanyam and Kolte, 1980).

The disease has been reported to cause a huge grain yield loss in Queens-land, Australia, where yield potential of 1.25 t/ha of the crop was reduced to 0.1 t/ha (Allen *et al*., 1981).

Sunflower is a crop of high commercial value but its yield is affected by several factor including diseases. *Alternaria* leaf blight is considered as a major disease and can cause yield losses from 15 to 90 % (Berglund, 2007).

The damages are due to the formation of leaf spots, reduction of plant photosynthetic area and early defoliation (Alves *et al*., 2013).

It has both pre and postharvest impact and has been found to cause 30-80% losses in seed yield and 17–33% reduction in oil content (Deokar *et al*., 2014).

Among the various diseases of sunflower, necrosis, leaf spot or blight occurs in early stages and powdery mildew which occurs in later stages are most important as they cause drastic yield losses (25-40 percent) under field conditions (Shankergoud *et al*., 2006; Chander Rao *et al*., 2015).

2.5. Symptom of *Alternaria* leaf blight of sunflower

The disease generally first appears on the lower leaves in the form of dark-brown to black spots measuring 0.2 to 0.5 mm in diameter. The circular to oval spots are surrounded by chlorotic zone with grey-white necrotic centre marked with concentric rings. The disease spread to middle and upper leaves with the growth of plants. In advanced stages, elongated spots are formed on petioles, stems and ray florets. Particularly under humid conditions, the spots enlarge in size and coalesce resulting in blighting of leaves and sometimes the flower heads are also infected (Amaresh and Nargund, 2001; Mukewar *et al.,* 1974).

The *Alternaria* leaf blight is known to infect all aerial parts of plant viz., leaf, petiole, stem, floral parts and seeds. Initially, the disease appears in the form of small, scattered, brown spots on the lamina. Later, these spots increase in size and coalesce covering larger leaf area (1.0 to 2.5 cm in diameter), with dark brown margin and yellow halo. Linear necrotic lesions also appear on stem, petioles and sepals. In severe cases, the head and seed also get infected (Tubaki and Nishihara, 1969; Narain and Saksena, 1973; Koltle and Mukhopadhyay, 1973 and Anilkumer *et al.*, 1974).

Severe infection of *Alternaria* blight of leaf, stem, petiole and inflorescence including petals have been described by many workers (Balasubramanyam and Kolte, 1980; Nargundand and Nazeer, 1994).

Hiremath *et al*. (1990) observed cracking of stem and petioles along with other symptoms in severely infected plants. Prathuangwong *et al*. (1991) observed that the pathogenic fungi can invade the discs, stems, and leaves of sunflower, causing round brown lesions, leaf drop, and even death of the plants.

Kalmesh (2011) isolated *Alternaria helianthi* from infected leaves of sunflower showing typical dark brown to black, circular to irregular spots by following standard tissue isolation technique.

Sunflower is most susceptible to *Alternaria helianthi* during anthesis and seed filling stage of growth. However, *Alternaria helianthi* can cause seedling blight, which reduces crop stand and can infect both leaves and stems of 10-32 days old plants. The disease symptoms appear more frequently on older leaves than on young and expanding ones (Devi *et al*., 2013).

2.6. Isolation and identification of *Alternaria alternata*

Keissler (1912) had given the morphology of *Alternaria alternata*. According to him the colonies were usually black or olivaceous black and sometimes grey. Conidiophores produced singly or in small groups, simple or branched, straight or flexuous, sometimes geniculate, pale to mid olivaceous or golden brown, smooth, up to 50 μm long, 3-6 μm thick, with one or several conidial scars. Conidia formed in long often branched chains, obclavate, pyriform, ovoid or ellipsoidal often with short conical or cylindrical beak sometimes up to but not more than one third the length of the conidium, pale to mid golden brown, smooth or verruculose with up to eight transverse and usually several longitudinal or oblique septa. Overall length 20-63 μm, 9-18 μm thick in the broadest part, beak pale, 2-5 μm thick.

Utikar and Padule (1980) reported that conidiophores of *Alternaria alternata* were light brown, simple, mostly 2-3 septate rarely 4-5 septate and variable in length ranging from 17.10 to 61.56 μm (Average 36.44 μm). Conidia were found to be light to dark brown, uniform with 1-6 transverse septa and 0-2 longitudinal septa, and variable in size and shape, mostly obclavate to oval with rudimentary beak and measured 10.26-77.52 × 4.56-14.82 μm (Average 42.45 × 10.27 μm).

Sundaresh and Hiremath (1981) isolated *Alternaria tenuissima* from necrotic area on infected leaf surface of soybean on potato dextrose agar.

Espinoza-Verduzco *et al*. (2012) reported that mycelium of *Alternaria alternata* was dark coloured; conidia were obclavate, obpyriform, ovoid or ellipsoidal, in chains, dark, large, with 1–7 transverse and 1–3 longitudinal septa, 13.97–57.5 × 6.35–20.32 µm.

Devi *et al*. (2016) recorded the maximum conidial length (62.16µm), width (15.60 µm), beak length (24.50 µm) and maximum number of conidial cells (3-8).

Prasad *et al*. (2020) shown that among the *A. alternata* isolates, the pigmentation varied from light green to light grey, dark grey to dark green, grey to light green and yellowish to light grey with light yellowish aerial mycelium. Conidia of *Alternaria alternata* were obpyriform, ovate to obclavate, yellowish-brown to brown, with 3–6 transverse and 1–5 longitudinal or oblique septa. Across the isolates, the range of average conidial length varied from 57 to 92 µm and width ranged from 24 to 39 µm. The average of transverse conidial septa ranged between 3 and 6.

2.6.1. Cultural Studies of *Alternaria* sp.

Neergaard (1945) showed that growth and sporulation of *A. solani* developed well in potato dextrose agar (PDA) medium.

Pawar and Patel (1957) reported that the growth of the pathogen was best on oat meal agar, while it was moderate on Richard's agar, potato dextrose agar and lima bean agar, sporulation was also profuse in Richard's agar.

Mukewar *et al*. (1974) observed poor growth but abundant sporulation of *Alternaria helianthi* on PDA. The isolate often showed conidia in chain of 2 to 3.

Agrawath *et al.* (1980) found oat meal agar as a good medium for virulent growth of *Alternaria helianthi*.

Joshi (1981) observed maximum growth of *Alternaria gomphrenae* on both potato dextrose agar and Richard's agar.

Mahabaleshwarappa (1981) recorded maximum growth of *Alternaria carthami* on potato dextrose agar followed by Richard's agar.

Aponte *et al*. (1988) observed poor growth of *Alternaria helianthi* on PDA. However, this medium helped in abundant sporulation after 8 days of infection at 24-28°C under dark condition.

Virendra *et al.* (2001) reported that best mycelial growth (51.1 mm) and sporulation of *Alternaria alternata* was observed on potato-dextrose agar (PDA) media followed by potato mashed dextrose agar, nutrient agar, host leaf extract agar and Richard's agar, Asthana and Hawker's agar, Haustan's agar, beef extracts agar, Czapek's Dox agar, V-8 juice agar and Elliot's agar media.

Waghunde *et al*. (2010) observed that potato dextrose agar (PDA) medium supported best mycelial growth and excellent sporulation of the *Alternaria alternata* (Fr.) Keissler followed by oat meal and corn meal agar. Highest dry mycelial weight was recorded in potato dextrose broth (586.5 mg) over all other media.

Devi *et al*. (2016) showed among different solid and liquid media, potato dextrose agar medium supported the fungus to attain the maximum growth of all the ten isolates of *Alternaria helianthi*.

Prasad *et al*. (2020) reported that *Alternaria alternata* grow well and sporulate profusely on PDA, however showed slow growth on sunflower leaf extract medium.

2.6.2. Pathogenicity

Ponnappa (1970) employed several inoculation techniques and reported that the symptoms appeared much earlier on the plants sprayed with suspension of spores and mycelium, following pricks made by needle, proving that the fungus enters through wounds much quicker than uninjured epidermis.

Anilkumer *et al.* (1974) reported that leaf spot of sunflower caused by *Alternaria helianthi* was pathogenic and on inoculation with conidial suspension to one

month old plants, symptoms appeared after 48 hours. Spots were first yellow and later turned brown with serrated margin, conidia were cylindrical to ellipsoid, pale brown with 1 to 12 septa.

Mukewar *et al*. (1974) reported that first symptoms of *Alternaria helianthi* on sunflower were noticed 24 hours after inoculating the leaves, in the forms of numerous light brown to brown fleck and oval to circular spots were elongated on streaks of desirables size.

Dhiman and Bonbale (1980) used suspension of pathogen culture containing 2000 spores/ml of distilled water for proving pathogenicity of *Alternaria solani* causing early blight of tomato. Further, they atomised the culture suspension on three leaf stage seedlings at the rate of 30 ml per seedling for successful inoculation.

Herr and Lipps (1982) reported that the leaf and stem spot of sunflower was first observed in Ohio. In severely affected field, many plants were defoliated and lodged. Under glass house condition, inoculations with suspension of 15,000 conidia/ml, host plant died. The pathogen was seed borne and over wintered in diseased sunflower residues. They reported that three isolates of *Alternaria helianthi* obtained from seed lots of three commercial sunflower cultivars, were pathogenic on four weeks old sunflower plant.

Allen *et al*. (1983a) reported that the amount of chlorosis induced by *Alternaria helianthi* in sunflower was greatest in plants that were inoculated at the vegetative or budding stage.

Godoy and Fernandes (1985) reported on inoculation of plant in glass house after 30, 45, 60, 75, 90, 105 days of sowing older plants and leaves were more susceptible to the pathogen than younger ones.

Lagopodi and Thanassoulopoulos (1996) tested pathogenicity of an isolate *Alternaria alternata* on sunflower and on twenty-five plants species including sunflower. Out of 4 botanical families of cultivated plants and 7 families of

weeds tested in the green house for susceptibility. Only sunflower was found highly susceptible.

Amaresh (1997) proved the pathogenicity on sunflower by inoculating spore Suspension of *Alternaria helianthi* (10^6 spores/ml) using automizer. The symptoms appeared 5-6 days after inoculation.

Prathibha (2005) proved the pathogenicity of *Alternaria helianthi* on sunflower by inoculating spore suspension (10 spores/ml) grown on PDA. The symptoms appeared 8-9 days after inoculation.

Prasad *et al*. (2008) reported that the sunflower leaf extract and carrot agar media supported the growth and sporulation of *Alternaria helianthi*. The germination capacity and infectivity of spores of *Alternaria helianthi* were lost upon continuous sub culturing up to 60 days after isolation. In pot culture experiments, the intensity of leaf spot was more on 25 and 30 days old plants, older plants (60 days old) failed to show disease symptoms. Increase inoculum concentration from 10^2 conidia to 10^6 conidia/ml significantly increased the infection. Disease intensity was at minimum (at 5×10^2 conidia/ml) for successful infection, an inoculum of 1×10^6 spores/ml and 25 to 30 days old plant ideal.

Udayashankar *et al*. (2011b) the inoculated plants were maintained in control climate room with a photoperiod of 12 hours light and 12 hours darkness at 22 ±2°C and 70-80% RH. The plants were observed for typical light/spot disease development on leaves and flower heads.

2.7. Role of weather factors in severity of disease

Tubaki and Nishihara (1969) noticed the first appearance of disease in the spring and increased rapidly during the rainy season.

Alternaria helianthi is a serious leaf spot pathogen of sunflower crop especially during kharif season i.e., monsoon grown sunflower crop (Kolte and Makhopadhyay, 1973; Narain and Saksena, 1973; Agrawat *et al.*, 1980; Herr and Lipps, 1981; Allen *et al*, 1983a, b).

Alternaria helianthi grows well between 18-30°C and most rapidly between 28-30°C. However, more conidia were produced at the lower end of the range (18-26°C). At least 12 h of 100 percent RH is required for infection of the host to take place (Islam and Maric, 1980).

Allen *et al*. (1982) reported that a 12h period of leaf wetness was required to give the maximum infection (lesions per square centimetre) at 25 and 28°C. Repeated periods of dew and high relative humidity promoted the expansion of lesions.

Alternaria helianthi attacks the leaves, stems and lowers of *Helianthus*. Since its optimal growth conditions require high temperatures (25-30°C) and high humidity, the infections are most severe in warm temperate to tropical areas (for example, Queensland (Australia), India, Yugoslavia and the southern USA) where sunflowers are widely grown as a crop (Allen *et al*., 1983a).

In 14-20 days old culture spore germination was optimum. Spores germinated well at 15°C-30°C and at 92.2 percent RH with pH of 6.0 to 6.6 (Somsundar and Anilkumer, 1987).

Hiremath *et al*. (1990) reported positive correlation between disease severity and relative humidity. The high humid conditions prevailed during rainy season caused disease epidemics in many parts of Karnataka.

Jasbir *et al*. (1991) reported that seed infection by *Alternaria* sp. was obviously favoured by the cool, damp conditions and heavier rainfall during flowering of the winter season crop.

Borkar and Patil (1995) reported that the temperature of 25.9°C to 33.7°C with a relative humidity of 89 to 95 percent favoured disease development.

Venkataramana *et al.* (1995) reported that the ideal temperature for *Alternaria helianthi* infection in sunflower is between 25-27°C and needs 12 hours wetness.

Kong *et al*. (1995) reported that the susceptibility of sunflower tissue increased with age, so that older leaves were more susceptible than young and expanding leaves. They also reported that planting density in field conditions also influenced the disease development.

Kumar and Singh (1997) studied the influence of weather factors on development of leaf spot of sunflower caused by *Alternaria helianthi* under field conditions during *kharif* 1990 and 1991. These results revealed that the most important weather factors favouring disease development were the temperature and relative humidity ranging from 27-29°C and 78-80% respectively.

Raranciuc (2002) stated that *Alternaria helianthi* shows optimum growth at 25-28°C. Conidial germination decreased with increasing temperatures above 25°C and reduced at temperatures below 20°C. Light had a positive influence, resulting in more rapid growth and abundant fructification of the fungus.

Versha *et al*. (2002) observed that the disease was severe in the month of july, with an average temperature of 29.78°C-30.31°C, relative humidity of 83.45-84.4% and rainfall of 60mm. Maximum disease was favoured by atmospheric temperature of 28°C- 31°C, relative humidity more than 88% and rainfall of 100-120 mm. Maximum susceptibility to infection was recorded at 60 days old plant sunflower.

Negative correlation between *Alternaria* leaf blight and mean maximum temperature was observed, while positive correlation was observed with rainfall and relative humidity (Amaresh *et al*., 2003).

Mondal *et al*. (2006) observed that high relative humidity 80%, optimum temperature (30%) and rain that prevail during June and July favoured the sporulation and development of the disease.

Prathibha *et al*. (2008) reported that the temperature of 25°C and pH of 6.5 had significant effect on the growth of *Alternaria helianthi*.

Silva and Freitas (2008) reported that the oil production capacity in different climate and soil conditions makes sunflower cultivation recommended for Rio Grande do Sul.

Mesta *et al*. (2009a) reported that the maximum temperature had significantly negative effect at 45, 60 and 75 DAS. However, minimum temperature had positive significant PDI. This explains the fact that, a moderate temperature is

very much congenial for the development of the disease. Both morning and evening relative humidity had positive significant effect on PDI during *khanif/ rabi* 2004-2005 at all the stage of crop growth, but in *kharif/rabi* 2005-2006 only morning relative humidity had positive significant effect. Rainfall had significantly positive effect on the disease development. However, its effect was more pronounced during *kharif/rabi* 2004-2005 than 2005-2006. This may be due to highly erratic pattern of the rainfall during *kharif/rabi* 2005-2006.

Within the diseases, *Alternaria* leaf spot or blight is a serious and potentially destructive one causing premature defoliation favoured by higher relative humidity and warm temperature (Prathuangwong et al., 1991; Wang et al., 2014).

2.8. Evaluation of plant extract against *Alternaria* sp.

Shekhawat and Prasad (1971) reported that out of nine plant extracts tested, five viz., *Allium cepa, A. sativum, Ocimum sanctum, Mentha piperita* and *Beta vulgaris* showed strong inhibitory action against *Alternaria* sp.

Srinivas *et al*. (1997) reported that foliar application of *Allium sativum* bulb extracts 1% concentration was significantly superior in controlling the disease and increase yield and was on per with carbendazim 1% a. i. KCL (soil application) and azadirectin also reduced the disease intensity and increased the yield over control.

Singh and Majumdar (2001) tested the plant extract of Neem, Dhatura, Tulsi and rhizome/bulb extract of ginger, turmeric, garlic and onion against *Alternaria alternata in vitro* by poisoned food method at 5, 10, 15, 20 % concentrations. The five extracts found effective *in vitro* were evaluated on pomegranate fruit as pre and post inoculation treatments. All the plant extracts at 20% concentration resulted in significant disease reduction but maximum reduction was observed with garlic extract followed by turmeric.

Poorniammal and Sarathambal (2009) found that the maximum reduction in the leaf spot disease caused by *Alternaria helianthi* was observed in plants sprayed

with propiconazole (83.38%) followed by neem oil (62.88 %) when compared with the control.

Mesta *et al*. (2009) found that among the plant extracts, neem leaf extract (38.49%) was effective than all other plant extracts with respect to inhibition of *Alternaria helianthi* spore germination on sunflower when compared to fungicides.

Ranaware *et al*. (2010) *in vitro* evaluated aqueous extract of seven plant species against *Altemaria cathami* causing leaf spot of safflower. Out of which *Allium sativum* recorded highest 48.68%, *Azadirachta indica* 28.73% and *Oscimum sanctum* recorded 25.029% radial growth inhibition.

Pareek *et al*. (2012) tested five different plant extract at 5%, 10% and 15% concentration against *Alternaria alternata* of cucumber. Out of which *Allium sativum* recoded 71.23% and *Azadirachta indica* recorded 57.10% mean mycelial growth inhibition.

Gholve *et al*. 2012) tested different plant extracts (5%, 10% and 15%) against *Alternaria macrospora* in cotton. Out of these Garlic extract recorded highest 37.479%, Tulsi 32.86% and Neem 25.28% mean mycelial growth inhibition.

Mishtra and Gupta (2012) used eight different plant extract at 10% concentration against *Altemarna porri.* Out of which *Allium sativum* recorded 58.05%, *Ocimum sanctum* 28.0% and *Azadirachta indica* 27.87% mycelial growih inhibition.

Chethana *et al*. (2012) tested six plant extract at different concentration (5%, 10%, 15% and 20%) against *Altermaria porri*. Garlic clove extract recorded 73.71% and Turmeic rhizome extract recovered 10.33% mycelial growth inhibition.

Sasode *et al*. (2012) evaluated six crude plant extract at (10%) against *Alternaria brasssicae* under *in vitro* condition. Out of which neem leaf, eucalyptus leaf and tulsi leaf recorded 20.73%, 23.15% and 32.12% mycelia growth inhibition over control.

Prasad *et al*. (2013) showed that among different concentrations tested, reduction in spore germination of *Alternaria helianthi* was recorded with 0.5 % of all plant extracts. Garlic extract was found to be effective with 85.1% reduction in spore germination over control followed by neem (83.3%) and *P. pinata* (75.9 %).

Devi *et al*. (2013) conducted an *in vitro* experiment to evaluated the growth of *Alternaria helianthi* causing leaf blight of sunflower against twenty plant leaf extracts viz., *Acalypha indica, Azadirachta indica, Alternanthera sessilis, Aloe vera,Vitex negundo, Wedelia calendulaceae, Centella asiatica, Ocimum tenuiflorum, Giliricidia maculate, Nila nirgundi, Leucas aspera, Lantana camera, Solanum trilobatum, Tephrosia purpurea, Hibiscus canabinus, Cissus quadrangularis, Mentha arvensis, Polyanthes tuberose, Polygala elata* and *Solanum xanthocarpurm* by poisoned food technique. Among them, leaf extracts of *Acalypha indica* at 10 per cent concentration inhibited the mycelial growth, sporulation and spore germination to about 78.38 per cent, 85.90 per cent and 52.48 percent respectively.

Devi *et al*. (2013) found that *Azadirachta indica* leaf extract was very effective against *A. helianthi* and can be used to manage this fungus under field condition.

Prasad *et al*. (2013) found that among the plant extracts, garlic showed disease reduction of 52.0% over pathogen check followed by neem.

Maya and Thippanna (2013) used eleven different plant extract at different concentration (10%, 20% and 30%) against *Alternaria solani* causing early blight of tomato. Out of which *Azadirachta indica* recorded 78.80% mean mycelial growth inhibition and *Eucalyptus globules* recorded 59.50%.

Taware *et al*. (2014) tested eleven plant extract (10%) against *Alternaria carthami* and observed that *Allium sativum* recorded highest 59.26%, *Curcuma longa* 42.96%, *Azadirachta indica* 24.07% and *Ociumum sanctum* 17.04% mycelial growth inhibition of *Alternaria carthami*.

Bhosale *et al*. (2014) tested different aqueous leaf extract against *Alternaria alternata* of soybean. Neem, ginger and eucalyptus were found to be highly inhibitory to mycelial growth of *Alternaria alternata*.

Kantwa *et al*. (2014) tested efficacy of seven plant extracts each at five concentrations 50, 100, 200, 500 and 1000 ppm against *Alternaria alternata* of groundnut. Garlic clove extract was found most effective in inhibiting the mycelial growth (46.60%) followed by leaf extract of neem (43.30%), tulsi (35.85%) and rhizome extract of ginger (34.48%).

Regmi *et al*. (2014) reported that leaf extract of *Jatropa curcas* showed maximum mycelial growth inhibition of *Alternaria alternata* (62.9%) followed by *Datura strumarium* leaf extract (55.6%) and *Azadirachta indica* (51.9%).

Murmu *et al*. (2015) tested four different plant extract against the *Alternaria solani*. Out of which *Azadirachta indica* recorded (52.42%) maximum mycelial growth inhibition.

Kansara and Sabalpara (2015) conducted an *in vitro* experiment of seven botanicals against *Alternaria alternata* causing leaf spot of niger. Among these effective botanicals, highest average mycelial growth inhibition was recorded with Garlic bulb extract 67.30% followed by leaf extract of Eucalyptus 60.089%, Neem 56.289%, Turmeric 54.375% and Tulsi 26.629%.

Waghe *et al*. (2015) conducted an *in vitro* experiment to evaluated five botanicals each at 10 and 20 % concentration against *Alternaria helianthi* caused leaf blight of sunflower by food poison technique. Among botanicals, maximum inhibition was recorded with Neem (63.05% and 68.88%) in addition to karanaj (56.38% and 63.60%) at 10 and 20% concentration.

Rakholia *et al*. (2016) reported that garlic cloves extract was highly inhibitory to the growth of *Alternaria alternata* causing ripe fruit rot of chilli followed by ardusi leaf extract and gave 58.96 and 33.83 percent inhibition respectively. Amina and Shamim (2016) tested antifungal properties of ethanol extracts of *Azadirachata indica*, *Allium sativum* and *Datura metel* at 5%, 10% and 20%

concentration against *Alternaria alternata* in chickpea and found that *Allium sativum* was most efficient inhibitor of the *Alternaria alternata* followed by *Datura metel* and *Azadirachta indica*.

Bugalia *et al*. (2017) tested Garlic leaf extract against *Alternaria brassicicola* and recorded 57.909% disease intensity as compare to control 60.62% under field condition.

Kumar *et al*. (2017) tested four different plant extract at 10% concentration against *Alternaria carthami* out of which Eucalyptus leaf extract recorded 32.23% and Neem leaf extract recoded 26.96% mean disease intensity over control under field condition.

Joseph *et al.* (2017) evaluated the efficacy of some leaf extracts in the management of early blight of tomato. The study showed that among the extracts tested, *Carica papaya* was the most effective in reducing the severity of early blight of tomato and significantly higher yields obtained with *Carica papaya*.

Dagadu (2017) showed that among the five botanicals, *Allium sativum* L. was found most effective inhibiting (53.33%) of the pathogen *Alternaria helianthi* followed by *Zingiber officinalis* L. (51.11%).

Rajhans and Sharma (2017) evaluated seven different plant extract at different concentration against *Alternaria alternata* causing core rot of Apple. Out of which *Azadirachta indica* recorded 84.80% mean mycelia growth inhibition.

Sunita *et al*. (2017) evaluated six different plant extract at 10%, 15% and 20% concentration against *Alternaria solani*. Out of which *Azadirachta indica* leaf extract recorded 42.11% mean mycelial growth inhibition and *Ocium sanctum* recorded 36.92% over control.

Jakatimath *et al*. (2017) evaluated different botanicals *in vitro* for control of *Alternaria alternata* and found that onion and garlic bulb extract inhibited maximum mycelial growth of *Alternaria alternata* both at 5 and 10 percent concentration.

Rani *et al*. (2018) reported that garlic clove extract at 10% concentrations exhibited the maximum (84.316) inhibition against *Alternaria alternata* whereas 82.18% inhibition of mycelial growth was observed in case of *Alternaria tenuissima* followed by onion and neem extract.

Kadam *et al*. (2018) tested eleven different plant extract at 10% concentration against *Alternaria alternata*. Out of which *Allium sativum* recorded highest 72.416, *Azadirachta indica* recorded 66.676%, *E. globules* 60.379% and *C. longa* recorded 52.96% mycelial growth inhibition.

Mahadevaswamy *et al*. (2019) found that among the botanicals neem seed kernel extracts at 10 percent (43.74%) showed positive effect on inhibiting of mycelium growth of *Alternaria sp.* followed by neem leaf extracts (25.26%).

CHAPTER III

MATERIALS AND METHODS

3.1. Experimental site

The laboratory experiment was conducted in Central MS laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University and the field experiment was conducted at Central Farm of Sher-e-Bangla Agricultural University, Dhaka-1207.

3.2. Experimental period

The experiment was conducted during the period from November 2019 to May 2020.

3.3. Materials

The following materials were used in the experiment

1) **Small tools** viz., scissor, needles, knife, blade, scale, forceps, cork borer, sprit lamp, electronic balance, wrapping tape, blotter paper, aluminium foil.
2) **Large equipments** viz., incubator, autoclave, laminar air flow, oven, refrigerator.
3) **Chemicals** viz., 70% ethanol, hexisol, agar powder, dextrose powder, lactic acid, glycerine, cotton blue.
4) **Glassware** viz., Petri dish, Funnel, Test tube, Beaker, Conical flask Slide, Cover slip.
5) **Data recording materials** viz., microscope, computer, camera.
6) **Others** viz., zip lock polythene bag, cotton cloth, potato, tissue paper, garlic bulb, neem leaf, mint leaf, allamanda leaf, papaya leaf, mahagony leaf.

3.4. Laboratory experiment

3.4.1. Collection of diseased specimens

Sunflower leaves showing typical symptoms of *Alternaria* blight were collected from Central Farm of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207 and kept in a zip lock polythene bags to maintain the moist condition.

3.4.2. Preparation of culture medium

Potato Dextrose Agar (PDA) medium was used for isolation, purification and preservation of the causal organism of leaf blight of sunflower.

PDA media was prepared according to standard protocol described by Ricker and Ricker (1936). The composition of PDA media is given below:

Ingredient	Quantities
Potato	200 g
Dextrose	20 g
Agar	20 g
Distilled Water	1 L

200 gm sliced, peeled potatoes were boiled in 1 liter distilled water to make potato infusion for 30 min. Potato infusion was filtered through cheese cloth and kept in a conical flask. Then 20g dextrose and 20g agar were added with potato infusion. Thoroughly, mixed All ingredient by using a glass rod and few amount of water was added to this solution to make the volume 1L. Then the mouth of the conical flask was closed by cotton plug and wrapped with aluminium foil paper. After that, the media was sterilized by autoclaving at 15 lbs. pressure at 121°C temperature for 15 minutes. Before pouring the media in petri dishes 1 ml acetic acid was added to the media under laminar airflow cabinet and adjusted pH 5.5 ± 0.2.

3.4.3. Isolation of *Alternaria* sp. from diseased leaf of sunflower by tissue planting method

Disease samples were cut into small pieces (2-2.5mm) having both disease and healthy tissue and kept in sterilized petri dish. Then, the cut pieces were surface sterilized with 70% ethanol for 60 seconds. After that, the cut pieces were transferred to a dish containing sterile water and washed thoroughly with two changes of sterile water to free from chemical. Three-layer water-soaked blotting paper were placed into the sterile petri dish for making moist chamber. Four small pieces of sample were transferred into the moist chamber and incubated at 25±2°C for 7 days in 12 hours with *alternata* light and darkness and observed daily. When the pathogen was grown in moist chamber then a bit of mycelia was taken with the help of sterilized needle and transferred on PDA plates aseptically. Then the plates were incubated in inverted fashion in an incubator at 27°C temperature. A profuse growth of a fungus on in plates was observed after 5 days of incubation (Plate 1). Then, it was repeatedly sub-cultured on potato dextrose agar medium to get pure culture of the organism.

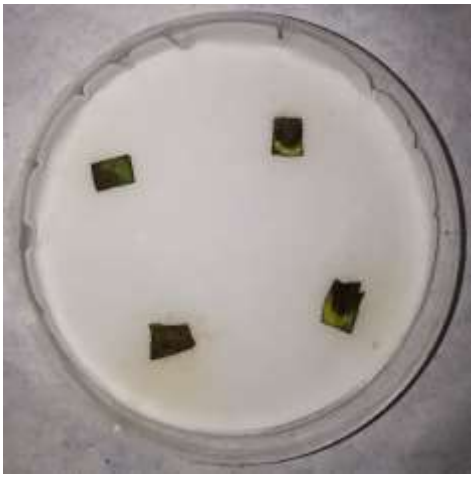
3.4.4. Identification of the causal organism

A slide was prepared from the pure culture of the organism and observed under the compound microscope. The organism was identified following the keys of Keissler (1912). This pathogenic isolate was stored in PDA slant for further studies.

3.4.5. Purification and maintenance of pure culture

When the pure culture of target fungus was achieved, 5 mm culture discs of the fungal mycelium were cut with the help of sterilized cork borer and transferred aseptically in potato dextrose agar slants and allowed to grow. The pure culture slants were sealed with paraffin wax and stored in a refrigerator at 4°C for further use.

(a) A portion of sunflower leaf showing typical leaf blight symptoms



(b) Incubation of pieces of diseased sample in moist chamber

(c) Initial growth of the pathogen on PDA media

Plate 1. Isolation of causal organism of leaf blight of sunflower by tissue planting method

3.4.6. Pathogenicity Test

Pathogenicity test (Plate 2) was carried out to establish the isolated fungus capability of producing typical symptoms of leaf blight under artificial inoculation condition on sunflower and re-isolate the pathogen to conform Koch's postulates.

Sunflower seeds (BARI Surjomukhi-2) were surface sterilized with 70% ethanol and sown in earthen pots containing sterilized soil and they were allowed to grow for a month. After one month, plants were thoroughly cleaned with sterilized distilled water using moist cotton. Later, the plants were sprayed with distilled water to sterilized the plant. 30 days old culture was taken which was grown on PDA medium and made 10^6 spores/ml suspension which was measured by haemocytometer. The conidial suspension was sprayed uniformly on the leaves. Control plants were also maintained by spraying with sterilized water.

The inoculated plants were covered with polythene bag to maintain high humidity for 2 days. Observation was made at regular intervals for the symptom development after 5 days of inoculation.

The symptoms first appeared at 7 days after inoculation. The organism was re-isolated from these artificial infected leaves showing leaf blight symptoms and the culture obtained was compared with the original culture for conformation.

(a) Seed sowing in sterilized soil

(b) Raising of healthy plant

(c) Spraying of conidial suspension (1×10^6 spores/ml)

(d) Plants were covered with polythene bags for maintaining high humidity

Plate 2. Preparation of different stages of pathogenicity test in sunflower

3.5. *In vivo* evaluation of plant extracts against *Alternaria alternata*

3.5.1. Climate

The experimental area was under the sub-tropical climate which characterized by the comparatively low rainfall, low humidity, low temperature, relatively short day during November to May and high rainfall, high humidity, high temperature and long day period during April to September.

3.5.2. Soil type

The soil of the experimental site belongs to the Agro-Ecological Zone of "Madhupur Tract" (AEZ No. 28). It was Deep Red Brown Terrace soil and belongs to "Nodda" cultivated series. The top soil is slightly clay loam in texture. Organic matter content was very low (0.82%) and soil pH varied from 5.47-5.63.

3.5.3. Variety selection and collection

BARI Surjamukhi-2 was selected based on susceptibility to *Alternaria* leaf blight disease and seed was collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur-1701.

3.5.4. Land preparation

A piece of medium high land with well drainage system was selected. The experimental field was first ploughed on 10th November 2019. The land was ploughed thoroughly with a power tiller and then laddering was done to obtain a desirable tilt. The clods of the land were hammered to make the soil into small pieces. Weeds, stubbles and crop residues were cleaned from the land. The final ploughing and land preparation was done on 25th November, 2019.

3.5.5. Application of fertilizers and manures

The following doses of fertilizers and manures were applied for the cultivation of sunflower recommended by BARI.

Fertilizers/Manures	Dose	
	Kg/ha	g/plot
Urea	180	81
TSP	150	68
MoP	120	54
Gypsum	120	54
Cow dung	8000	3600

The 1/3rd urea and whole amount of other fertilizers were applied during final land preparation as basal dose and rest 2/3rd urea was applied at 30 DAS and 50 DAS followed by irrigation.

3.5.6. Design of the experiment

The experiment was conducted in randomized complete block design (RCBD) with three (3) replications and seven (7) treatments.

3.5.7. Layout preparation

The field layout was done as per experimental design on 30th November, 2019. The field was divided into three blocks each of which representing a replication. The unit plot size was 2.5m × 1.8m and plot to plot distance was 0.5m and block to block distance was 0.75 meter (Appendix-II).

3.5.8. Seed rate and seed sowing

Seed rate was 8-10 kg/ha. After layout preparation, seed was sown in line on 1st December, 2019.

3.5.9. Spacing

Seed of sunflower were sown maintaining row to row distance of 50 cm and plant to plant distance of 25 cm.

3.5.10. Intercultural Operation

Different intercultural operations were done as follows.

Irrigation

Irrigation was done at 7-10 days interval as per necessity.

Weeding

Weeding was done fourth time in the experimental period starting from 20 DAS, 30 DAS, 40 DAS and 55 DAS.

Thinning and gap filling

Extra plants were removed at 25 DAS by keeping a healthy plant per hill to maintain optimum population number. Gap filling was also done as per necessary.

3.5.11. Treatments details

Sl. No.	Treatments	Scientific name	Active ingredient	Plant parts used	Concentration (W/V)
T_1	Control	--	--	--	--
T_2	Neem leaf extracts	*Azadirachta indica*	Azadirachtin	Leaf	1:3
T_3	Garlic bulb extracts	*Allium sativum*	Allicin	Bulb	1:3
T_4	Mint leaf extracts	*Mentha piperita*	Menthol	Leaf	1:3
T_5	Allamanda leaf extracts	*Allamanda cathartica*	Allamandin	Leaf	1:3
T_6	Papaya leaf extracts	*Carica papaya*	Papain	Leaf	1:3
T_7	Mahogany leaf extracts	*Swietenia macrophylla*	Swietenin	Leaf	1:3

3.5.12. Collection of plant parts for preparing extracts

Leaves of neem, papaya allamanda and mahogany were collected from Sher-e-Bangla Agricultural University campus and garlic and mint were collected from Mohammadpur Krishi Market, Dhaka and presented in Plate 3.

(a) Neem leaf

(b) Garlic bulb

(c) Mint leaf

(d) Allamanda leaf

(e) Papaya leaf

(f) Mahogany leaf

Plate 3. Different plant parts used as treatment which were collected from SAU campus and Mohammadpur krishi market

3.5.13. Preparation of plant extracts

The extracts were prepared by following the method of Ashrafuzzaman and Hossain (1992). For preparation of extracts, fresh leaves were weighted in an electronic balance and then washed in the water. After washing, the large leaves were cut into small pieces. For getting extract, weighted plant parts were blended and added with distilled water. The pulverized plant tissue was squeezed through 3 folds of fine cotton cloth. For getting 1:3 (w/v) ratio, 300 ml of distilled water was added with 100g plant parts (Plate 4).

Plate 4. Extracts of different plant parts

3.5.14. Application of plant extracts

The plant extracts were applied in the field as foliar spray. Spraying was done at 3 times at 15 days interval which starting from vegetative growth at 30 DAS, 45 DAS and 60 DAS. Precautions were taken to avoid drifting of spray materials from plant to neighbouring plants.

3.5.15. Disease of the plants

Natural infection of the plants was considered in this experiment.

3.5.16. Data collection

Five plants were randomly selected from each of the plot and the data was recorded on the following parameters

1) Disease incidence (%)
2) Percent disease index
3) Plant height (cm)
4) Number of leaves per plant
5) Stem girth (cm)
6) Head diameter (cm)
7) Number of seeds per head
8) 1000 seeds weight (g)
9) Yield (g/plot)

Disease Incidence

Data recorded on disease incidence at 45 DAS, 60 DAS and 75 DAS after the appearance of visible symptoms of leaf blight of sunflower. Disease incidence was calculated from the number of infected leaves on a plant against the total number of leaves existed at the time of observation which was express in percentage. Thus, formula of disease incidence was

$$Disease\ incidence\ (DI) = \frac{No.of\ infected\ leaves}{Total\ no.of\ leaves\ observed} \times 100$$

Percent disease index

Percent disease index (PDI) was also recorded at 45 DAS, 60 DAS and 75 DAS to quantify amount of infection. It was calculated as per the 0 to 9 disease ratting scale (Plate 5) developed by Mayee and Datar (1986). For this purpose, two

leaves at the bottom, two middle and two top of the plant were chosen as per scale given below:

Leaf area disease (LAD)	Score	Disease reaction
1) Zero	0	Immune
2) Less than 1%	1	Highly resistant
3) 1-5%	3	Resistant
4) 6-25%	5	Moderately resistant
5) 26-50%	7	Susceptible
6) more than 50%	9	Highly susceptible

The average plant disease index of each plot was worked out by using following formula

$$PDI = \frac{Sum\ of\ total\ disease\ rating}{Total\ no.of\ observation\ \times Highest\ grade\ in\ the\ scale} \times 100$$

PDI reduction

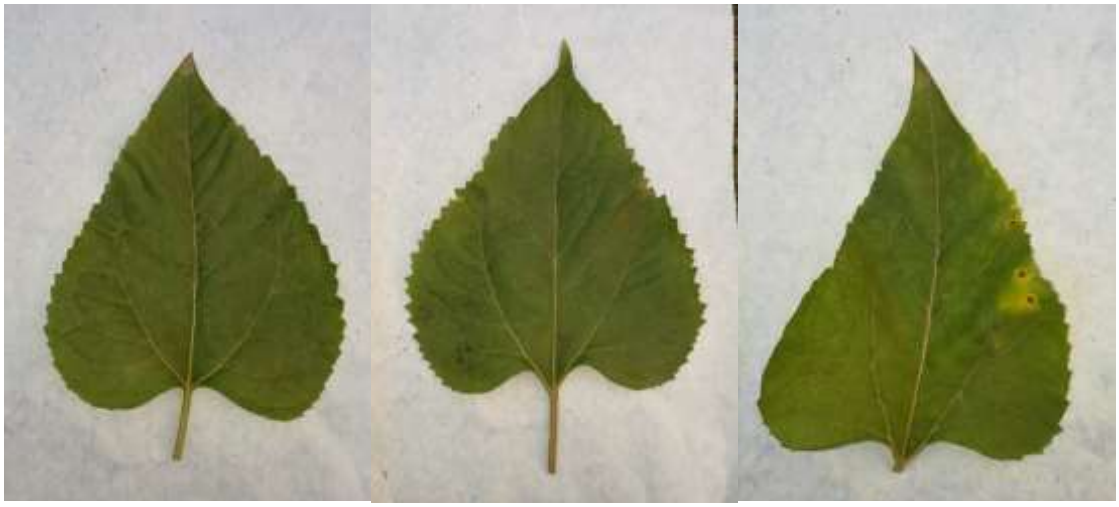
PDI reduction over control was calculated by using the following formula and expressed in percentage

$$PDI\ reduction\ over\ control = \frac{C - T}{C} \times 100$$

Where,

C = PDI in Control

T = PDI in Treatment



LAD = 0% | LAD = <1% | LAD = 1-5%

Score 0 | Score 1 | Score 3

LAD = 6-25% | LAD = 26-50% | LAD = >50%

Score 5 | Score 7 | Score 9

Plate 5. Assessment of *Alternaria* leaf blight of sunflower according to disease rating scale

Plant height (cm)

Plant height was measure from the base of the plant to the point of attachment of the capitulum at 75 DAS and express in centimetres.

Number of leaves per plant

The number of fully opened leaves from the base to the tip of the terminal bud was counted at 75 days after sowing.

Stem girth

The stem girth was measured at the middle point of the stem at 75 DAS.

Head Diameter (cm)

Head diameter was measured when the capitulum (Head) of the plant is fully mature at 90 DAS.

Harvesting and threshing

The selected sunflower plants were harvested at 105 DAS when all plants were fully matured. Yield of each treatment in all replications were recorded separately.

Number of Seeds per Head

Total number of seeds in a head was counted by manually after threshing.

1000 seeds weight (g)

1000 seeds were randomly selected and separated from each of the treatment of all replication and weighted by using mini electronic balance at 12% seed moisture.

Yield

Heads were harvested, sundried and threshed separately with all treatment of all replication. Finally, yield data was recorded on the basis of plot size.

Percent increase in yield over control

Percent increase in yield over control was calculated by using the following formula

$$Percent\ increase\ in\ yield\ over\ control = \frac{T - C}{C} \times 100$$

Where,

C = Yield in Control

T = Yield in Treatment

3.5.17. Statistical analysis

For statistical analysis, the recorded data were compiled, tabulated and subjected in Microsoft Excel 2019. Analysis of variance (ANOVA) and LSD at 5% were done by using computer package program Statistix 10.0.

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Symptomatology

Symptoms on the leaf surface were observed under field conditions in the form of small circular, brown coloured spot on the surfaces of leaves. As the disease progressed, these brownish spots increased in size and finally coalesced to cover the entire surface of the leaves with dark brown margin and yellow halo with distinct zonation's producing blight symptoms. The blighted leaf finally gets curled and became dark blackish in colour. Marked Blight symptoms are seen in the head (capitulum) of heavily infected plants in which involucres and ray florets are distinctly blighted. Blight symptoms are the result of continuous necrosis in which the infecting fungus has killing effect on invaded tissue (Plate 6).

These symptoms were similar to the symptoms studied by Tubaki and Nishihara, 1969; Narain and Saksena, 1973; Kolte and Mukhopadhyay, 1973 and Patel *et al.*, 2010.

According to Tubaki and Nishihara (1969) the *Alternaria* leaf blight is known to infect all aerial parts of plant viz., leaf, petiole, stem, floral parts and seeds. Initially, the disease appears in the form of small, scattered, brown spots on the lamina. Later, these spots increase in size and coalesce covering larger leaf area (1.0 to 2.5 cm in diameter), with dark brown margin and yellow halo. Linear necrotic lesions also appear on stem, petioles and sepals. In severe cases, the head and seed also get infected.

(a) Symptom first appeared as small circular, brown color spots

(b) Brownish spots increased in size

(c) Spots coalesced with dark brown margin and yellow halo with distinct zonations

(d) Head also affected in severely infected plant

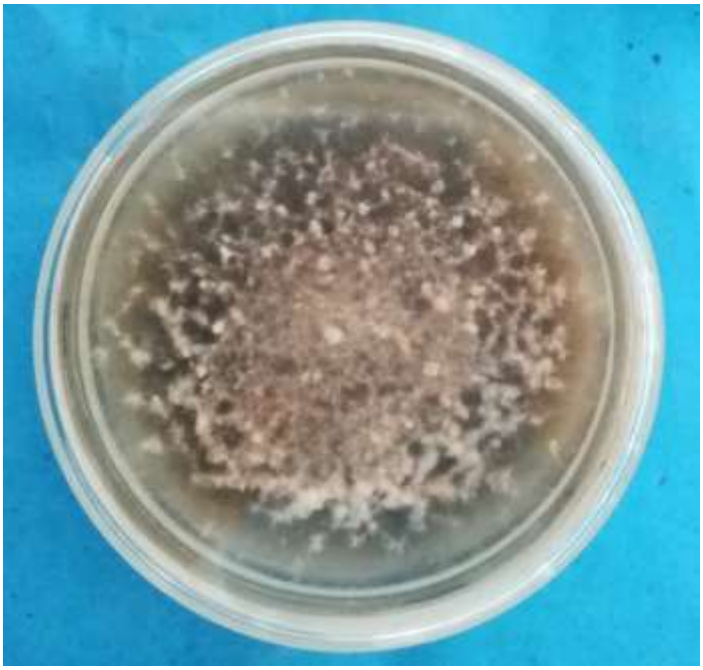
Plate 6. Symptoms of leaf blight of sunflower at different stage of plant growth

4.2. Isolation and identification of fungus

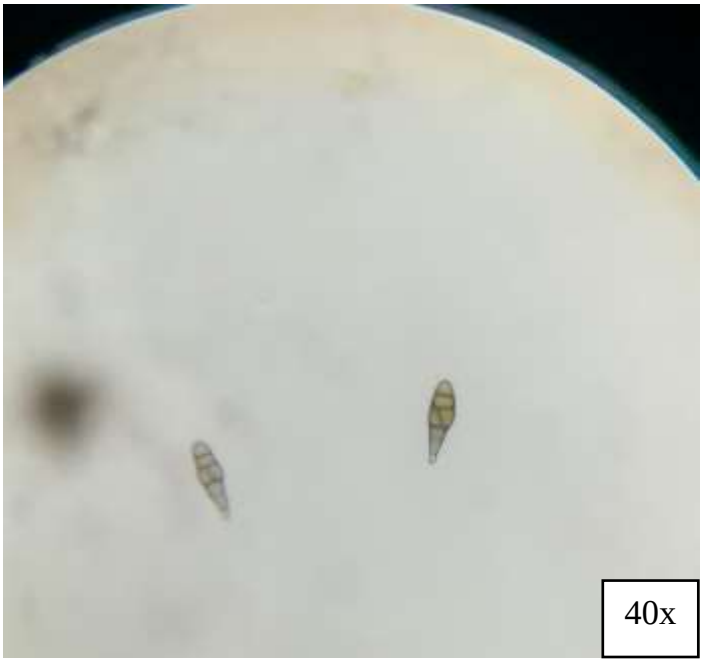
Isolation of the pathogen was made from sunflower leaves showing typical symptoms of the disease. The fungus was successfully isolated on Potato dextrose agar medium and obtained profuse growth and maximum sporulation. In pure culture the fungal colony was initially white, cottony with profuse aerial mycelium which gradually turned greenish grey (Plate 7). Aged culture appeared completely black with no aerial mycelium. Conidiophores were short to long, simple or branched arising singly. Conidiophores were hyaline to golden brown coloured. Conidia were observed to arise either singly or in chains at the tip of each conidiophore. Conidia are typically muriform, dark brown, thick walled, in long chains (9-15). Majority of conidia are non-beaked few with short rudimentary dark brown beaks, 6 - 7 transverse septa and 0 - 3 longitudinal septa (Plate 8). Based on the characters of the colony and morphological characters of conidiophores and conidia the fungus was identified as *Alternaria alternata.*

Similar kinds of results have been reported by several researchers (Prasad *et al*., 2020; Devi *et al*., 2016; Espinoza-Verduzco *et al*., 2012 and Keissler, 1912).

According to Keissler (1912) the colonies were usually black or olivaceous black and sometimes grey. Conidiophores produced singly or in small groups, simple or branched, straight or flexuous, sometimes geniculate, pale to mid olivaceous or golden brown, smooth, up to 50 µm long, 3-6 µm thick, with one or several conidial scars. Conidia formed in long often branched chains, obclavate, pyriform, ovoid or ellipsoidal often with short conical or cylindrical beak sometimes up to but not more than one third the length of the conidium, pale to mid golden brown, smooth or verruculose with up to eight transverse and usually several longitudinal or oblique septa. Overall length 20-63 µm, 9-18 µm thick in the broadest part, beak pale, 2-5 µm thick.

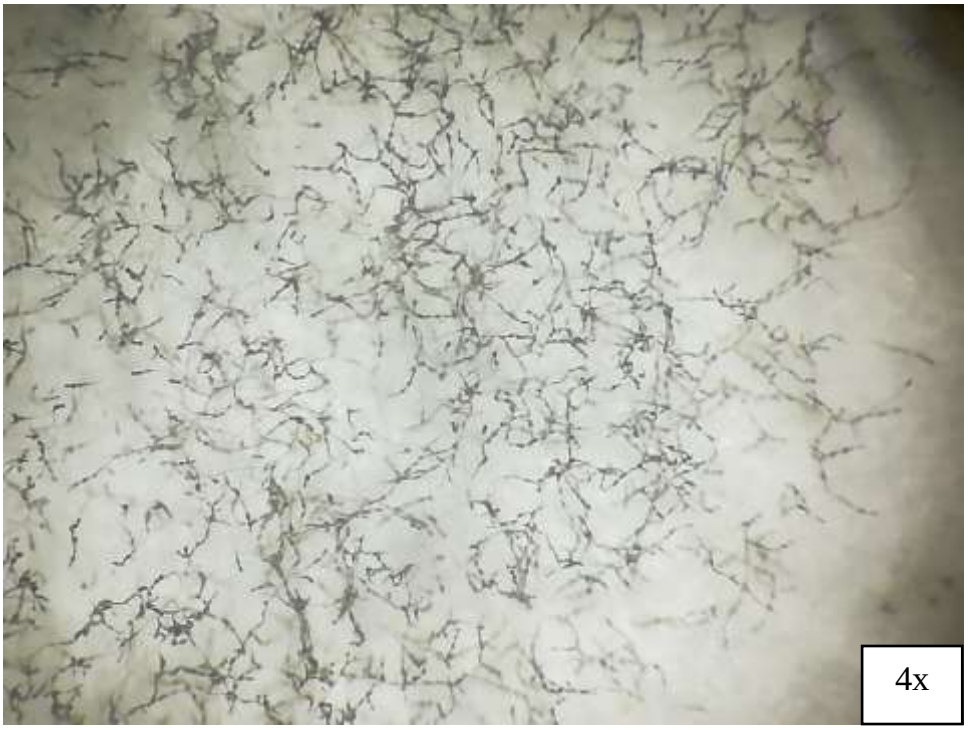


(a) Pure culture of *Alternaria alternata*



(b) Muriform conidia of *Alternaria alternata*

Plate 7. Identification of *Alternaria alternata* as a causal organism of leaf blight of sunflower



(b) Stereo microscopic view

(a) Compound microscopic view

Plate 8. Conidial chain of *Alternaria alternata* under different microscope

4.3. Pathogenicity test

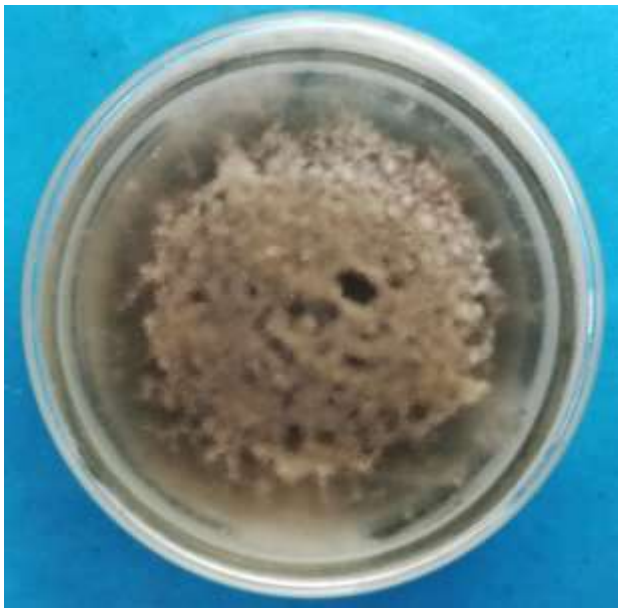
The typical symptoms like small scattered brown spots appeared on the leaf surface. Later, the spots increased in size, covering large area with dark brown margin and yellow halo and distinct zonation was noticed on leaves of the artificially inoculated plants (Plate 9).

The pathogens were re-isolated from such leaves and the morphological character of the re-isolated organism was compared with the original culture of the pathogen which was similar in all respects. Hence, the causal agent of the disease was confirmed as *Alternaria alternata*.

The similar observations were reported by Lagopodi and Thanassoulopoulos (1996) tested the pathogenicity of an isolate *Alternaria alternata* on sunflower and on twenty-five other plant species, including members of 4 botanicals families of cultivated plants and 7 families of weeds in the green house for susceptibility by artificial inoculations. Of the plants tested, sunflower was the only highly susceptible species. Similarity Prathibha (2005) reported the pathogenicity on sunflower by inoculating spore suspension of *Alternaria helianthi* (10^6 spores/ml) grown PDA. The symptoms appeared 8-9 days after inoculation.

(a) No symptom developed because of no inoculation of pathogen

(b) Symptoms developed result of inoculation of pathogen



(c) Re-isolated of the pathogen

Plate 9. Confirmation of pathogenicity of *Alternaria alternata* in sunflower

4.4. Effects of different plant extracts on disease incidence of leaf blight of sunflower at 45, 60 and 75 DAS

The effect of selected plant extracts on disease incidence of leaf blight of sunflower is presented in Table 1. All treatments showed promising performance in reducing the disease incidence at different days after sowing (DAS).

At 45 DAS, the highest disease incidence (40.96 %) was recorded in T_1 (Control) followed by T_4 (Mint leaf extracts @ 1:3 w/v) 33.03% and T_2 (Neem leaf extracts @ 1:3 w/v) 32.37%. The lowest disease incidence (26.87%) was recorded in T_3 (Garlic bulb extracts @ 1:3 w/v) followed by T_7 (Mahogany leaf extracts @ 1:3 w/v) 27.82%, T_6 (Papaya leaf extracts @ 1:3 w/v) 28.97% and T_5 (Allamanda leaf extracts @ 1:3 w/v) 31.43%.

At 60 DAS, the lowest disease incidence (40.50%) was also recorded in case of T_3 (Garlic bulb extracts @ 1:3 w/v) followed by T_2 (Neem leaf extracts @ 1:3 w/v) 43.13%, T_6 (Papaya leaf extracts @ 1:3 w/v) 44.47% and T_5 (Allamanda leaf extracts @ 1:3 w/v) 46.20% leaf. The highest disease incidence (64.92%) was recorded in T_1 (Control) followed by T_7 (Mahogany leaf extracts @ 1:3 w/v) 55.88 and T_4 (Mint leaf extracts @ 1:3 w/v) 50.20.

At 75 DAS, the least disease incidence (43.32%) was observed in T_3 (Garlic bulb extracts @ 1:3 w/v). T_6 (Papaya leaf extracts @ 1:3 w/v) gave disease incidence 47.38% followed by T_2 (Neem leaf extracts @ 1:3 w/v) 50.07%. The highest disease incidence (81.01%) was recorded in T_1 (Control) followed by T_7 (Mahogany leaf extracts @ 1:3 w/v) 73.86% and T_4 (Mint leaf extracts @ 1:3 w/v) 71.82%.

These findings were partially supported by Mesta *et al*., 2009; Prasad *et al.,* 2013; Devi *et al.,* 2013; Mahadevaswamy *et al.,* 2019; Ravinder *et al*., 2020.

Prasad *et al*. (2013) reported that garlic bulb extract was found to be effective with 85.1% reduction in spore germination over control followed by neem (83.3%) and *P. piñata* (75.9 %.). *A. indica* leaf extract was very effective against

A. helianthi and can be used to manage this fungus under field condition (Devi *et al*., 2013).

Mesta *et al*. (2009) found that among the plant extracts, neem leaf extract (38.49%) was effective than all other plant extracts with respect to inhibition of *Alternaria helianthi* spore germination on sunflower when compared to fungicides. Neem seed kernel extracts at 10 percent (43.74%) showed positive effect on inhibition of pathogen mycelium growth followed by neem leaf extracts where the value was 25.26% (Mahadevaswamy *et al*., 2019).

Ravinder *et al*. (2020) evaluated four plant extracts against *A. solani*. Among them neem leaf extract at 15% (w/v) was found to be most effective in inhibiting the mycelial growth of the pathogen with inhibition of 52.36 %, followed by combination of garlic clove and green chilli extract with the inhibition of 50.42%.

Table 1. Effect of different plant extracts on disease incidence of leaf blight of sunflower at 45, 60 and 75 DAS

Treatments	Disease Incidence (%)		
	45 DAS	60 DAS	75 DAS
T_1=Control	40.96 a	64.92 a	81.01 a
T_2=Neem leaf extract	32.37 b	43.13 de	50.07 d
T_3=Garlic bulb extract	26.87 d	40.50 e	43.32 e
T_4=Mint leaf extract	33.03 b	50.20 c	71.83 b
T_5=Allamanda leaf extract	31.43 bc	46.20 cd	59.40 c
T_6=Papaya leaf extract	28.97 bcd	44.13 de	47.38 de
T_7=Mahogany leaf extract	27.82 cd	55.88 b	73.86 b
CV (%)	7.68	5.81	4.68

*Values in a column with same letter (s) do not differ significantly (p=0.05)

4.5. Effects of different plant extracts on percent disease index (PDI) of leaf blight of sunflower at 45, 60 and 75 DAS

Effect of different plant extracts viz., neem, garlic, mint, allamanda, papaya and mahogany were evaluated at different days after sowing on percent disease index (PDI) of leaf blight of sunflower (Table 2). The results revealed that all the treatments were found significantly superior over control at different days after sowing (DAS).

At 45 DAS, the maximum (24.84%) PDI was noted in control (T_1) followed by mint, neem and papaya leaf extracts where PDI were 21.88%, 19.38% and 17.74%, respectively. The minimum (12.84%) PDI was recorded in garlic bulb extracts (T_3) followed by mahogany leaf extracts (T_7) and allamanda leaf extracts (T_5).

At 60 DAS, Garlic bulb extracts (T_3) was shown minimum (19.54%) PDI and it was significantly lower than all other treatments. Allamanda leaf extracts (T_5) gave second minimum PDI 23.31% followed by mahogany leaf extracts (T_7) and neem leaf extracts (T_2). The maximum (35.96%) PDI was recorded in control (T_1) proceed by mint leaf extracts (T_4) and papaya leaf extracts (T_6).

At 75 DAS, the least 26.12% PDI was also recorded in case of garlic bulb extracts (T_3) followed by papaya leaf extracts (T_6) and neem leaf extracts (T_2) resulting 28.49% and 29.29%, respectively. Treatments T_3, T_6 and T_2 were statistically similar. The highest 48.05% PDI was recorded in control proceed by mahogany leaf extracts (T_7) and mint leaf extracts (T_4).

Among the treatments, the maximum 45.64% disease reduction at 75 DAS was recorded in garlic bulb extracts (T_3) followed by papaya leaf extracts (T_6) and neem leaf extracts (T_2) resulting 40.71% and 39.09%, respectively. The minimum 17.86% disease reduction was observed in mahogany leaf extracts (T_7) followed by mint leaf extracts (T_4) and allamanda leaf extracts (T_5) is presented in Figure 1.

The result agrees with the result of previous research workers (Babu *et al*., 2000; Singh and Singh, 2007; Singh and Verma, 2010; Prasad *et al*., 2013).

Babu *et al*. (2000) tested some plant extracts and plant oils against tomato leaf blight (*Alternaria solani*) under glasshouse and field conditions and found that sprayed with 3% neem oil on tomato resulted in 53% reduction in disease severity over control.

Singh and Singh (2007) recorded the maximum disease control when the plants treated with *A. indica* (42.3%) followed by *L. camara* (38.6%), *L. inermis* (36.1%), *D. metel* (33.3%), *C. procera* (27.3%) and *C. medica* (19.1%) against *Alternaria* blight of linseed.

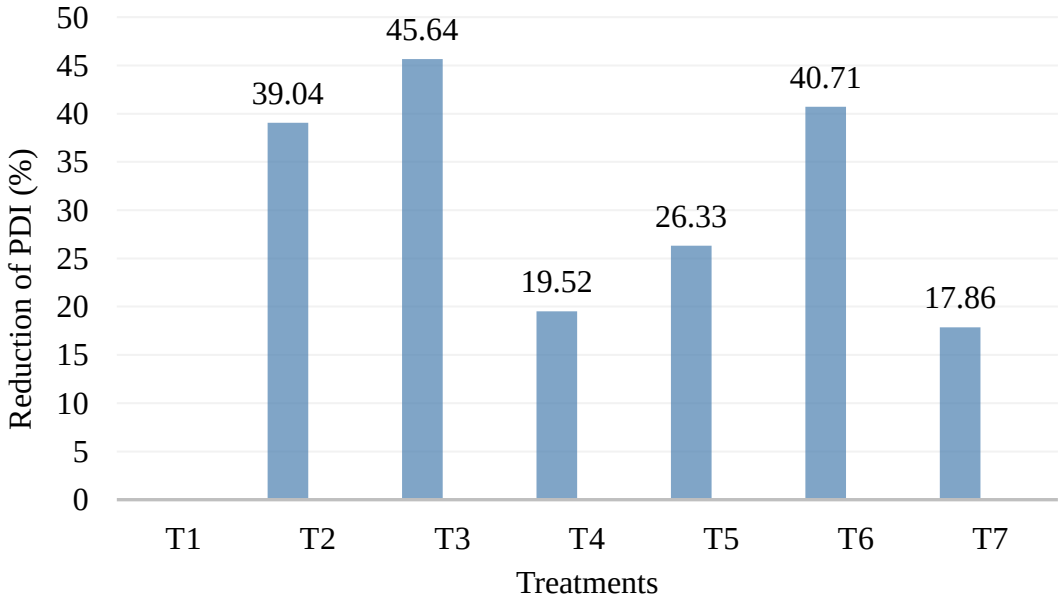
Singh and Verma (2010) reported that garlic extract proved as the most effective treatment in checking growth and conidial germination of *Alternaria alternata* and also in controlling *Alternaria* leaf blight in Adusa. Two sprays of garlic extract at 15% gave 55.1% disease control on artificially inoculated Adusa plants.

Prasad *et al*. (2013) reported that among ten plant extracts, garlic (*A. sativum*) extract significantly reduced disease severity by 48 to 52% over check.

Table 2. Effects of different plant extracts on percent disease index (PDI) of leaf blight of sunflower at 45, 60 and 75 DAS

Treatments	Percent Disease Index (PDI)		
	45 DAS	60 DAS	75 DAS
T_1=Control	24.84 a	35.96 a	48.05 a
T_2=Neem leaf extract	19.38 bc	25.47 cd	29.29 d
T_3=Garlic bulb extract	12.84 f	19.54 e	26.12 d
T_4=Mint leaf extract	21.88 b	29.73 b	38.67 bc
T_5=Allamanda leaf extract	16.23 de	23.31 d	35.40 c
T_6=Papaya leaf extract	17.74 cd	26.54 bc	28.49 d
T_7=Mahogany leaf extract	14.63 ef	24.62 cd	39.47 b
CV (%)	7.90	6.80	6.19

*Values in a column with same letter (s) do not differ significantly (p=0.05)



Here, T_1=Control, T_2=Neem leaf extracts, T_3=Garlic bulb extracts, T_4=Mint leaf extracts, T_5=Allamanda leaf extracts, T_6=Papaya leaf extracts, T_7=Mahogany leaf extracts

Figure 1. Efficacy of plant extracts in reducing PDI of leaf blight of sunflower caused by *Alternaria alternata* at 75 DAS

4.6. Effect on different plant extracts on growth and growth contributing parameters of sunflower

Effect of different plant extracts on growth and growth contributing parameters like plant height, number of leaf per plant and stem girth was recorded at 75 DAS and the data was presented in Table 3.

Plant height

Among different treatments of plant extracts, the tallest (132.4 cm) plant was recorded in T_3 (Garlic bulb extracts) followed by T_2 (Neem leaf extracts) 127.63 cm and T_6 (Papaya leaf extracts) 126.08 cm. The effect of treatments in T_2 and T_6 were statistically similar. The Shortest (114.34) cm plant was recorded in T_1 (Control) proceed by T_7 (Mahogany leaf extracts) 114.03 cm and T_4 (Mint leaf extracts) 119.91 cm.

Number of leaves per plant

Among different treatments of plant extracts, the maximum number of leaves (28) were recorded in garlic bulb extracts treated plots T_3 followed by neem leaf extracts (T_2) and papaya leaf extracts (T_6) and the minimum number of leaves (24) were recorded in control plots T_1. All treatments were statistically similar in terms of number of leaves per plant without control.

Stem girth

Among different treatments of plant extracts, the thickest (5.47 cm) plant was recorded in garlic bulb extracts (T_3) followed by neem leaf extracts (T_2) and papaya leaf extracts (T_6) resulting 5.16 cm and 5.08 cm, respectively. The thinnest (4.02 cm) plant was recorded in control (T_1) followed by mahogany leaf extracts (T_7) and mint leaf extracts (T_4).

Table 3. Effect on different plant extracts on growth and growth contributing character of sunflower

Treatments	Plant Height (cm)	No. of Leaf per Plant	Stem Girth (cm)
T_1=Control	114.34 d	25 b	4.02 e
T_2=Neem leaf extract	127.63 b	28 a	5.16 b
T_3=Garlic bulb extract	132.40 a	28 a	5.47 a
T_4=Mint leaf extract	119.91 c	26 a	4.41 d
T_5=Allamanda leaf extract	120.55 c	27 a	4.73 c
T_6 =Papaya leaf extract	126.08 b	27 a	5.08 b
T_7=Mahogany leaf extract	117.70 cd	26 a	4.31 d
CV (%)	1.80	4.23	2.07

*Values in a column with same letter (s) do not differ significantly (p=0.05)

4.7. Effect on different plant extracts on yield and yield contributing parameters of sunflower

Effect of different plant extracts on yield contributing parameters viz., head diameter (cm), number of seed per head, 1000 seed weight were evaluated and presented in Table 4.

Head diameter (cm)

The maximum head diameter (17.78 cm) was obtained with the application of garlic bulb extracts followed by papaya leaf extracts (16.65 cm) and neem leaf extracts (16.46 cm) and the minimum head diameter (14.19 cm) was recorded in control followed by mahogany leaf extracts (14.74 cm) treated plot.

Number of seed per head

The maximum number of seeds per head (523) was recorded in garlic bulb extracts treated plot (T_3) followed by papaya leaf extracts and neem leaf extracts where the number of seeds per head were 486 and 472, respectively. The minimum number of seeds per head (340) was recorded in control plot followed by mahogany leaf extracts treated plot.

1000 seeds weight (g)

Among different plant extracts, garlic bulb extracts (T_3) was recorded highest 1000 seeds weight 57.46 g followed by neem leaf extracts (T_2) and papaya leaf extracts (T_6) 5.48 g and 54.61 g respectively. The lowest 1000 seed weight 47.15 g was obtained in treatment T_1 (Control).

Yield (g/plot)

The highest 778.87 g yield per plot was recorded in case of T_3 where garlic bulb extracts @ 1:3 w/v was applied as foliar spray. Treatment T_6 (spray with mahogany leaf extracts @ 1:3 w/v) was produced the second highest yield (722.65 g) followed by treatment T_2 (spray with neem leaf extracts @ 1:3 w/v) and T_5 (spray with allamanda leaf extracts @ 1:3 w/v) yielding 721.98 g and 672.85 g per plot, respectively. Treatment T_6 and T_2 were statistically similar in terms of yield. The lowest yield per plot (476.70 g) was noted in T_1 (Control).

Table 4. Effect on different plant extracts on yield and yield contributing parameters of sunflower

Treatments	Head Diameter (cm)	No. of Seed per Head	1000 Seed Weight (g)	Yield (g/plot)
T_1=Control	14.19 d	340 e	47.15 e	476.70 d
T_2=Neem leaf extract	16.46 b	472 bc	55.48 ab	721.98 b
T_3=Garlic bulb extract	17.78 a	523 a	57.46 a	778.87 a
T_4=Mint leaf extract	15.05 c	397 d	50.19 d	651.06 c
T_5=Allamanda leaf extract	16.08 b	437 c	51.99 cd	672.85 bc
T_6=Papaya leaf extract	16.65 b	486 ab	54.61 bc	722.65 b
T_7=Mahogany leaf extract	14.74 cd	371 de	49.77 de	631.52 c
CV (%)	2.54	4.80	2.86	4.60

*Values in a column with same letter (s) do not differ significantly (p=0.05)

4.8. Effect of different plant extracts on percent yield increased over control against *Alternaria alternata* causing leaf blight of sunflower

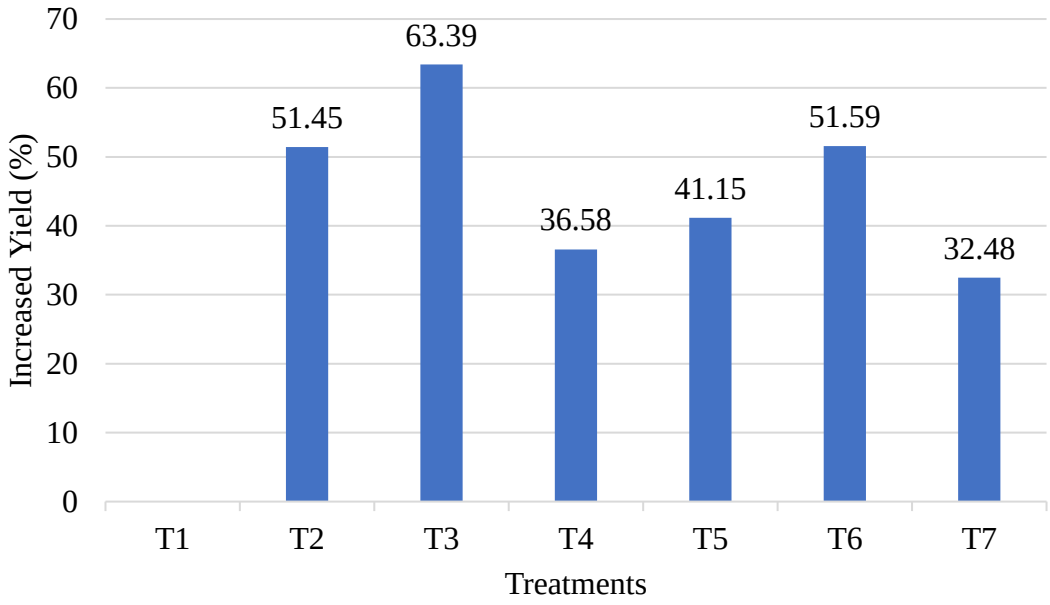
Effect on different plant extracts on percent yield increased over control against *Alternaria alternata* causing leaf blight of sunflower was evaluated in field condition and shown in Figure 2.

The maximum (63.39%) yield increased over control was found in case of the treatment T_3 (spray with garlic bulb extracts @ 1:3 w/v) followed by T_6 (spray with mahogany leaf extracts @ 1:3 w/v), T_2 (spray with neem leaf extracts @ 1:3 w/v) and T_5 (spray with allamanda leaf extracts @ 1:3 w/v) resulting 51.59%, 51.45% and 41.15%, respectively. The lowest yield increased was observed in T_7 (spray with mahogany leaf extracts @ 1:3 w/v) 32.48% preceded by T_4 (Mint leaf extracts @ 1:3 w/v) 36.58%.

The findings of this studies were also observed by several workers (Patil *et al*., 2001; Singh and Singh, 2007; Waghe *et al*., 2015; Zahid, 2016; Ravinder *et al*., 2020).

Patil *et al*. (2001) found that severity of early blight of tomato caused by *A. solani* was reduced by neem seed extract which also increased the fruit yield. *Azadirachta indica* increased yield 28.3% over control on *Alternaria* blight of linseed (Singh and Singh, 2007).

Waghe *et al*. (2015) reported that Minimum disease control (45.25%) was recorded in seed treatment with Neem seed powder at 10 g/kg + two sprays of Neem extract at 10% at 30 and 45 DAS with 908 kg/ha yield. Zahid (2016) reported that among the plant extracts the maximum yield increased was observed with Papaya leaf extracts (80.92%) followed by marigold leaf extracts (75.32%) and Neem leaf extracts (67.04%) against *Alternaria solani* causing early blight of tomato. Ravinder *et al*. (2020) found that neem leaf extracts increase yield 37.70% with decreasing PDI 61.34% over control.



Here, T_1=Control, T_2=Neem leaf extracts, T_3=Garlic bulb extracts, T_4=Mint leaf extracts, T_5=Allamanda leaf extracts, T_6=Papaya leaf extracts, T_7=Mahogany leaf extracts

Figure 2. Effect of different plant extracts on percent yield increased over control against *Alternaria alternata* causing leaf blight of sunflower

CHAPTER V

SUMMERY AND CONCLUSION

Sunflower (*Helianthus annuus* L.) is an important oil seed crop which is originated from Central and North America. A wide range of uses of sunflower have been reported throughout the world such as ornamental plant, medicinal, alimentary, feedstock, fodder, dyes for textile industry, body painting, decorations, and so on. Successful production of sunflower is mainly hindered by *Alternaria* leaf blight which is the most devastating disease, causing huge loss to growers. Several effective pesticides have been recommended for the control of *Alternaria* leaf blight of sunflower but they are not considered to be long term solutions, due to concerns of expense, exposure to heal the risk, fungicidal residue and other environmental hazards.

Considering the above mention fact, the present study was conducted to evaluated the effectiveness of some selected plant extracts against the pathogen of the leaf blight disease of sunflower. The pathogen was isolated by tissue planting method and identified by cultural and morphological characters given by Keissler (1912). Final confirmation was done by testing Koch's postulates. The treatments were consisted on plots basis viz., T_1=Control, T_2=Neem leaf extracts, T_3=Garlic bulb extracts, T_4=Mint leaf extracts, T_5=Allamanda leaf extracts, T_6=Papaya leaf extracts, T_7=Mahogany leaf extracts. Concentration of all plant extracts was 1:3 w/v. Three spray were done at 15 days intervals at 30, 45 and 60 days after sowing (DAS). Naturally infection of disease was considered in this experiment. Data were collected on disease incidence, percent disease index (PDI), plant height, number of leaves per plant, stem girth, head diameter, number of seeds per head, 1000 seeds weight and yield.

In pure culture the fungal colony was initially white, cottony with profuse aerial mycelium which gradually turned greenish grey and aged culture appeared completely black with no aerial mycelium. Conidiophores were short to long,

simple or branched arising singly. Conidia were observed to arise either singly or in chains at the tip of each conidiophore. Conidia are typically muriform, dark brown, thick walled, in long chains (9-15). Majority of conidia are non-beaked few with short rudimentary dark brown beaks, 6-7 transverse septa and 0-3 longitudinal septa. Based on the characters of the colony and morphological characters of conidiophores and conidia, the fungus was identified as *Alternaria alternata.*

The effect of different plant extracts on disease incidence and percent disease index (PDI) were varied significantly compared to control. At 75 days after sowing (DAS), the minimum disease incidence (43.32%) with minimum PDI (26.12) was recorded in garlic bulb extracts (T_3). Second lowest disease incidence and PDI were recorded in papaya leaf extracts (T_6) followed by neem leaf extracts (T_2). In all DAS, maximum disease incidence and Maximum PDI were recorded in control (T_1).

Among the plant extracts, the maximum disease reduction (45.64%) was observed in garlic bulb extracts (T_3) followed by papaya leaf extracts (T_6) and neem leaf extracts (T_2) and the minimum disease reduction (17.86%) was recorded in mahogany leaf extracts (T_7) followed by mint leaf extracts (T_4) at 75 DAS over control.

In terms of growth, yield and yield contributing parameters, tallest plant (132.4 cm), maximum number of leaves (28), thickest plant (5.47 cm), maximum head diameter (17.78 cm), highest number of seeds per head (523), 1000 seeds weight (57.46 g) and yield (778.87 g/plot) were also recorded in garlic bulb extracts (T_3). Neem leaf extracts (T_2) was recorded 127.63 cm plant height, 28 leaves per plant, 5.16 cm stem girth, 16.46 cm head diameter, 572 seeds per head, 57.46 g of 1000 seeds weight and yield (721.98 g/plot) followed by papaya leaf extracts (T_6). Lowest result was recorded in control of all growth, yield and yield contributing parameters.

The highest (63.39%) yield increase was recorded in garlic bulb extracts (T_3). The nearest yield increase was 51.59% recorded from the foliar application of

papaya leaf extracts (T_6). The seed yield 51.45%, 41.15%, 36.58% and 32.48% were increased by the foliar application of neem, allamanda, mint and mahogany leaf extracts, respectively.

Thousands of phytochemicals have inhibitory effect on several microorganisms. Considering the performances of plant extracts evaluated in field experiment it can be concluded that either, garlic bulb extract or papaya leaf extracts or neem leaf extracts with 1:3 w/v concentration can be used as eco-friendly management of leaf blight of sunflower. It was also suggested to carry out the study for several consecutive years in different Agro Ecological Zones (AEZs) to formulate a sustainable approach.

CHAPTER VI

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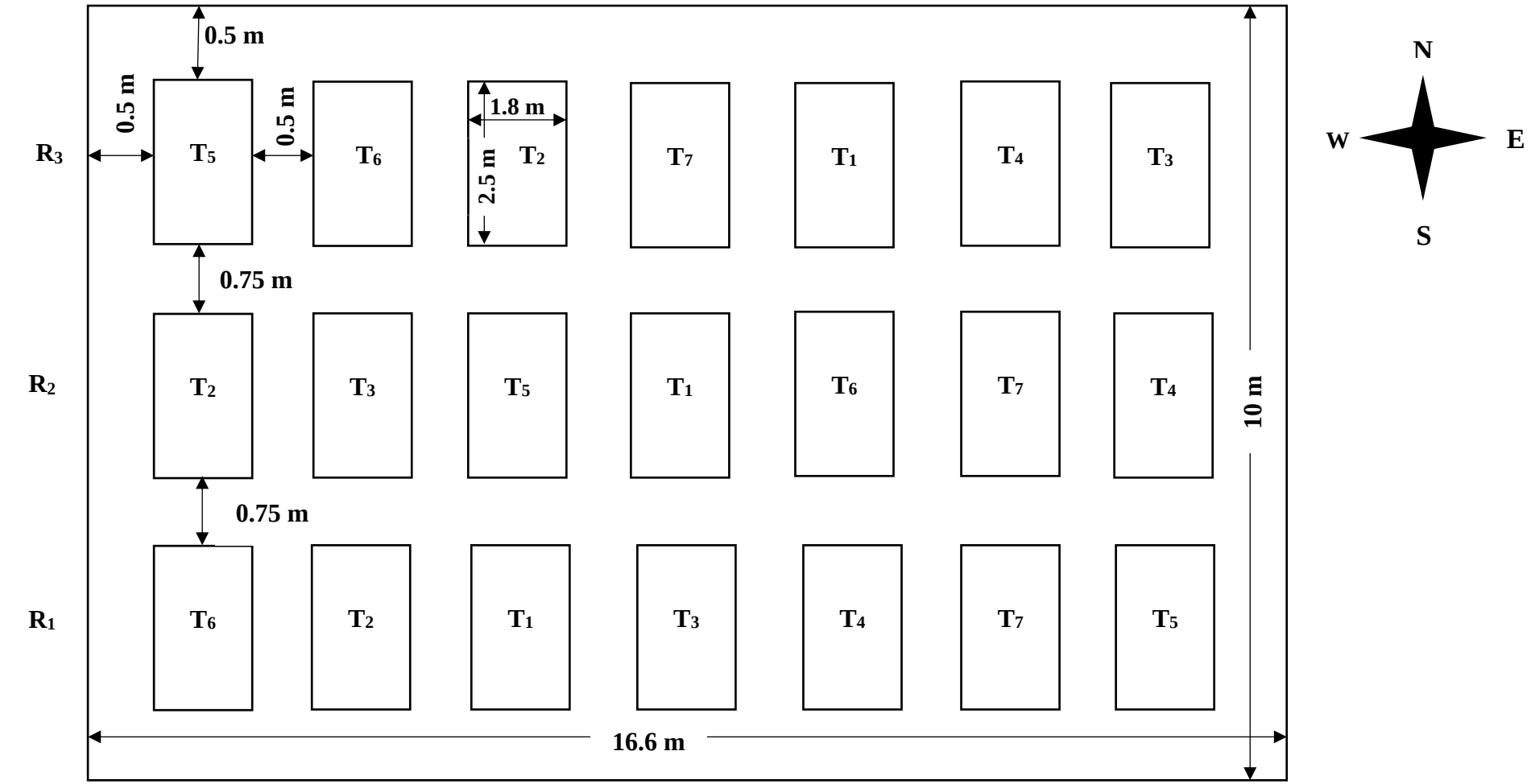
CHAPTER VII

APPENDIECS

Appendix I. Details of field experiment

1. Name of Crop	Sunflower
2. Variety	BARI Surjamukhi-2
3. Season	*Rabi* 2019-20
4. Design of Experiment	RCBD
5. No. of Treatments	7
6. No. of Replication	3
7. No. of Total Plots	21
8. Total Plot Size	166 m. sq.
9. Individual Plot Size	2.5m × 1.8m
10. Spacing	50cm × 25cm
11. Seed Rate	8-10 kg/ha
12. Sowing methods	Line sowing
13. Date of Sowing	01-December-2019
14. Date of Spraying	31-December-2019, 15-January-2020 and 30-January-2020
15. Date of initiation of disease	25-December-2019
16. Date of harvesting	15-March-2020

Appendix II. Layout of field experiment



Appendix III. LSD value for different parameters at 5% level of significance

Parameters	LSD $_{(0.05)}$
Disease incidence at 45 DAS	4.32
Disease incidence at 60 DAS	5.09
Disease incidence at 75 DAS	5.08
PDI at 45 DAS	2.56
PDI at 60 DAS	3.19
PDI at 75 DAS	3.86
Plant height	3.92
No. of leaves per plant	2.01
Stem girth	0.17
Head diameter	0.71
No. of seeds per head	36.91
1000 seeds weight	2.67
Yield	54.41

Appendix IV. Monthly maximum and minimum temperature, average relative humidity, rainfall, average sun hour and average wind speed of the experimental period (December 2019 to March 2020)

Year	Month	Average Temperature (°C)		Average RH (%)	Total Rainfall (mm)	Average Sun hour	Average wind speed (kmph)
		Max.	Min.				
2019	December	28	20	74	5	10.7	8.4
2020	January	27	18	76	21	10.7	8.3
	February	30	19	59	1	10.8	8.2
	March	35	24	57	30	11.6	9.5

Source: Bangladesh Meteorological Department (Climate division), Agargaon, Dhaka-1207.

Appendix V. Spraying of different plant extracts on the leaf surface of plant