SYMPTOMATOLOGY AND ETIOLOGY OF LEAF SPOT DISEASE OF GILOY

(*Tinospora cordifolia* Thunb.)

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A

Thesis submitted to the Odisha University of Agriculture and Technology in partial fulfilment of the requirement for the degree of

Master of Sciences in Agriculture

(Plant Pathology)

By

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CERTIFICATE – I

This is to certify that the thesis entitled **"Symptomatology and Etiology of Leaf Spot disease of Giloy (*Tinospora cordifolia* Thunb.)"** submitted in partial fulfilment of the requirement for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY)** to the **Odisha University of Agriculture and Technology** is a faithful record of bonafide and original research work carried out by **Riya Raju, Adm. No. 191222217** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

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

13.09.2021

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CHAIRMAN
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CERTIFICATE – II

This is to certify that the thesis entitled **"Symptomatology and Etiology of Leaf Spot disease of Giloy (*Tinospora cordifolia* Thunb.)"** submitted by **Riya Raju, Adm. No. 191222217** to the **Odisha University of Agriculture and Technology,** Bhubaneswar in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY)** has been approved by the students' advisory committee and the external examiner.

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Place: Bhubaneswar Riya Raju

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SYMBOLS AND ABBREVIATIONS

%	: Percentage
^{o}C	: Degree Celsius
"	: inches
AICRP	: All India Coordinated Research Project
AYUSH	: Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy
BOD	: Bio-Oxygen Demand
cm	: Centimeter
COVID-19	: Coronavirus Disease 2019
DMAPR	: Directorate of Medicinal and Aromatic Plants Research
et al.	: et alia (= and others)
Fig.	: figure
FYM	: Farm Yard Manure
Gms/Litre or g/L	: Grams per Litre
i.e.	: that is
kcal/mol	: kilo calorie per mol
Kg	: kilogram
m	: meter
MAP	: Medicinal and Aromatic Plants
ml	: millilitre
mm	: millimeter
NA	: Nutrient Agar
NBPGR	: National Bureau of Plant Genetic Resources
NICRA	: National Innovations in Climate Resilient Agriculture
NMPB	: National Medicinal Plants Board
PDA	: Potato Dextrose Agar

pH	: Soil reaction
Pvt. Ltd	: Private Limited
SARS-CoV-2	: Severe Acute Respiratory Syndrome Coronavirus-2
Sq. cm	: Square centimeter
SMPB	: State Medicinal Plants Board
t	: tonnes
TC	: Tinospora cordifolia
TSM	: Traditional Systems of Medicine
µm	: micrometer
viz.	: videre licet (= which is)
YASARA	: Yet Another Scientific Artificial Reality Application

ABSTRACT

Giloy (*Tinospora cordifolia*) is one of the most important medicinal plant with various anti-inflammatory and antipyretic properties. It is also recommended as one of the important prophylactic care measures against Corona (SARS-CoV-2) virus. These plants grow extensively in different states of India, including Odisha. Only few diseases have been reported in the herb in which majority are foliar. These foliar diseases cause severe damage to the medicinal herb resulting in deterioration of its phytochemical constituents and medicinal value along with drastic reduction in yield. Keeping in view the importance of the leaf spot diseases occurring in Giloy which adversely affects the shrub, the present investigation was carried out with a perspective to study the symptoms of leaf spot disease predominantly occurring in and around Bhubaneswar, Odisha and to detect, identify and characterise its causal organism. Samples from all the places of collection were more or less similar with little variations. The leaf spots that appeared on the plant were circular to irregular in shape with light grey to ash centre having dark brown margins. These spots were more evident on the ventral side of leaves while the corresponding dorsal side were yellow in colour. Later, the spots coalesced to form larger blighted areas which many a times covered the entire leaf surface and lead to total chlorosis, drying and defoliation of leaves. The present description of symptoms of the leaf spot disease is regarded as a new encounter. Pathogenicity test of isolates from leaf samples collected from different places were done and out of 15 isolates only 9 isolates produced the symptoms similar to that of original sample. Cultural and morphological characterisation of the virulent isolates were done. On PDA culture plates, all the isolates had white fluffy aerial mycelial growth initially and greyish black mycelium later, with little variations. The microscopic studies divulged the hyphae that was hyaline, multi-celled, septate, irregularly branched, and thin (2.94 µm in diameter) initially and later thickened (4.5 µm in diameter) and changed to light grey in colour. The fungus produced obclavate to muriform, beaked, golden-brown conidia with 2-3 transverse septa and several longitudinal septa borne in chains on straight, erect, septate, golden-brown conidiophores. Based on the cultural and morphological characterisation, the fungus was identified as ***Alternaria alternata*** and was further confirmed with the help of mycologists from the Department of Plant Pathology, OUAT, Bhubaneswar. No earlier worker has reported the infection of Giloy by *Alternaria alternata*, hence, to the best of my knowledge it is a new report. An effort was also made to determine most suitable medium for appreciable growth and sporulation of *Alternaria alternata* in the laboratory, using five different culture media. Among all the media used for study, the PDA was found to be the best support for mycelial growth of the fungus as well as its sporulation. It was followed by Richard's Agar in both the aspects. No earlier work reported nutritional requirement of *Alternaria alternata* causing leaf spot of Giloy. The present work therefore, is bound to cut the first turf for future workers.

INTRODUCTION

India is home to diverse range of medicinal and aromatic plants which have been the mainstay of traditional health care practices across all societies for centuries. Medicinal plants form the base of our indigenous health care tradition across the globe. A significant population includes medicinal plants and its products in their primary health care. Thus, the medicinal plants have become an integral part of people's life (Pandey, 2018). The State of Odisha has been a treasure house of many indigenous medicinal plants. A total of 2727 species with medicinal properties are available in Odisha, out of them only 166 species are cultivated plants whereas others are forest plants (Swain and Kumar, 2018).

Tinospora cordifolia, is one of the highly valued herbs in Indian medicine. It finds its application in the treatment of cancer, asthma, diabetes, blood pressure, skin diseases, and allergic conditions in Ayurveda, homeopathy and traditional systems of medicine (Rajakumar and Shivanna, 2009). Also, few recent studies have suggested that Giloy and Ashwagandha have a significant role to play in the treatment of novel Coronavirus infection (SARS-CoV-2), the causal agent of ongoing pandemic (Vellingiri *et al*., 2020). It is commonly known as heart-leaved moonseed, guduchi or giloy and is a large, glabrous, succulent and deciduous climbing shrub of the family menispermaceae. The plant is indigenous to tropical Indian subcontinent, but also found in Sri Lanka, Myanmar and China. The plant grows well in almost every kind of soil at different climatic conditions. Giloy is described as "one who protects the body against disease" in many ancient books and Vedas, validating its importance from age-old times.

The plant stem is greyish brown-black in colour from outside and light green in colour from inside and have long filiform aerial roots emerging and extending from branches. The stem is soft wooded, porous, dry, cylindrical and 5-25 mm in diameter (Dwivedi *et al*., 2014). Leaves are simple, alternate and exstipulate, with petioles up to 15 cm in length, bearing roundish and pulvinate leaves at apex and basal region. The plant grows to a height of 3-4 feet and is about 1 foot in width. It is one of the most versatile rejuvenating herbs and promotes longevity (Gupta *et al*., 2011). Several bioactive compounds including berberine, tinosporin, tinocordifolin and

tinosporafuranol from stem, choline and isocolumbin from roots, and protoberberine and tinosporol from leaves, have been reported (Ahmad *et al*., 2010). Giloy is also used as an appetizer, febrifuge, stomachic, cardiotonic, anthelmintic and adaptogen. The roots and stem of *Tinospora cordifolia* are also advised as an antidote in scorpion sting and snake bite.

T. cordifolia grows extensively throughout Indian tropical areas, mainly, the states such as Arunachal Pradesh, Assam, Bihar, Delhi, Gujarat, Goa, Karnataka, Kerala, Maharashtra, Odisha, Sikkim, Tamil Nadu, Uttar Pradesh and West Bengal (Ahmad *et al*., 2014). Warm and humid climate present in Odisha, along with an annual rainfall of 1200 mm to 1500 mm favours plant growth in the state. The plant is of great significance in the field of Ayurveda and Homeopathy, and also among common people of the state because of its immense medicinal benefits. Through recent observations and studies conducted on the plant, it is seen that many foliar diseases occur in the plant, that at times result into its premature defoliation and death. This leads to reduction of substantial yield from the shrub and affects the quality of bioactive compounds extracted from different plant parts.

Occurrence of foliar diseases caused by bacterium *Xanthomonas campestris* (Achar *et al*., 2014) and fungus *Phoma putaminum* (Shivanna *et al*., 2013) were observed in plants growing in Bhadra Wildlife sanctuary in Western Ghats region of Karnataka. In addition to these diseases, a new flat stem disease of giloy was reported recently, caused by Phytoplasma (Achar *et al*., 2015).

Leaf spot disease is generally characterised by initial appearance of small, yellow spots that turned into dark brown to black lesions surrounded by yellow halo and is a commonly used descriptor for identifying many plant diseases. These discoloured spots or lesions later develop a centre of necrosis or follow cell death. These symptoms can be caused by fungus, bacteria or virus or by injuries from nematodes, insects, environmental factors, toxicity or herbicides. Prolonged wet and humid conditions generally promote leaf spot disease, as greater number of pathogens are spread by wind, splashing rain or irrigation that carry the disease to other leaves. Although, leaf spot diseases affect a small percentage of host leaves, more severe consequences of leaf spot disease results in moderate to complete loss of leaves.

Leaf spots caused by fungi occur mostly due to the necrosis of plant tissues. These necrotic lesions, localised in area and shape, consist of dead and collapsed cells of the host leaves. One distinct feature of fungal infections is that there may be visible spores in the centre of leaf spots. Fungal leaf spots often have a black, brown, tan or reddish centre with a darker margin and differ in size.

Bacterial leaf spots occur as necrotic, circular or angular lesions and may have a yellowish outline or halo. Early symptoms of bacterial leaf spots are found on older leaves and lesions appear water-soaked. The most obvious symptom of bacterial leaf spots is the blackening of the spots after infection. Eventually older lesions dry out and become papery in texture. Bacterial spots can also produce white, yellow, light cream or silver bacterial exudates depending on the type of bacteria, which may ooze from splitting lesions or from the underside of the spots.

Contemplating the significance of different leaf spot diseases occurring in Giloy which adversely affects one of the most important medicinal shrubs of India as well as Odisha, and realizing that there is no report of any diseases on Giloy from Odisha, a systemic investigation was carried out in the Department of Plant Pathology, College of Agriculture, Bhubaneswar and AICRP on MAP and Betelvine, Bhubaneswar, where emphasis was given to the study of symptoms of leaf spot disease and to detect, identify and characterise the causal organism of leaf spot disease in *T. cordifolia*.

Keeping in consideration the above facts, the present study has been undertaken with the following objectives:

OBJECTIVES:

1. Describe the symptoms of leaf spot disease in *Tinospora cordifolia*.
2. Detection, characterization, identification of causal organism.
3. To prove pathogenicity of the isolated pathogen.

REVIEW OF LITERATURE

The review of literature covers about the medicinal plant *Tinospora cordifolia*, commonly referred to as Giloy and its medicinal properties and also how the plant has taken over a significant role during COVID-19 pandemic period. The emphasis is given to different biotic stresses occurring in plants with greater focus on leaf spot diseases. The review is being presented under the following headings 1) Distribution of *Tinospora cordifolia* 2) Importance and Utilisation 3) Status of Crop in India and Odisha 4) Challenges in successful production of Giloy 5) Biotic stresses in Giloy 6) Diseases in medicinal plants caused by *Alternaria alternata.*

2.1 Distribution of *Tinospora cordifolia*

Tinospora cordifolia, commonly known as heart-leaved moonseed or giloy is a herbaceous vine of the family Menispermaceae and is indigenous to Indian subcontinent but also found in Sri Lanka, Myanmar and China. It grows well in almost every kind of soil at different climatic conditions and can be easily grown as a rainfed crop. The plant is an abundantly spreading climbing shrub and attains height having several elongated coiling branches (Sinha *et al.*, 2004).

Out of 40 species of giloy distributed in tropical Africa, South- East Asia, Indo Malaya region and Australia, only three species are found in India namely *Tinospora cordifolia*, *Tinospora sinensis* and *Tinospora crispa* (Lohra and Kumar, 2019). In India, the plant grows extensively in states such as Arunachal Pradesh, Assam, Bihar, Delhi, Gujarat, Goa, Karnataka, Kerala, Maharashtra, Odisha, Sikkim, Tamil Nadu, Uttar Pradesh and West Bengal (Ahmad *et al*., 2014). In Odisha, Giloy is mostly found in forest and tribal areas in addition to waste and barren lands of the state (Swain and Kumar, 2018).

The plant has got many vernacular names in different languages and different regions *viz*., in Odisha it is called Guluchi, in Sanskrit Guduchi, in Malayalam Chittamruthu etc. (Khan *et al*., 2016; Upadhyay *et al*., 2010). These names have been presented in the Table 1.

Table 1. The Vernacular names* of Giloy (*T. cordifolia*)

Sl. No.	Language	Name
01.	**Arab**	**Gilo**
02.	Assamese	Hoguni
03.	Bengali	Guloncho
04.	**English**	**The nectar of immortality, Heavenly elixir**
05.	Gujarati	Galac, Garo
06.	Hindi	Giloy, Gurach, Gulvel
07.	Kannada	Amrita balli
08.	Konkani	Amritvel
09.	Malayalam	Chittamruthu
10.	Manipuri	Ningthou Khongli
11.	Marathi	Guduchi
12.	**Nepal**	**Gurjo**
13.	Odia	Guluchi
14.	Punjabi	Gllow
15.	Sanskrit	Guduchi, Amritha, Cinnodbhava
16.	**Sinhalese**	**Rasakinda**
17.	Tamil	Shindilakodi
18.	Telugu	Tippa-teega
19.	**Urdu**	**Gurch, Guluncha**

*Names in Foreign languages and English have been presented in bold font.

2.2 Importance and Utilization

Giloy is considered as an important "medicinal plant species in high trade" in India and is extensively harvested in wild (Ved and Goraya., 2007). This plant has also been listed in 178 medicinal plant species in High Volume Trade by NMPB, New Delhi, India (Mittal *et al*., 2014). The plant possesses potential medicinal properties to cure various human ailments and disorders. Thus, the plant got its name 'Amrita', which refers to 'the heavenly elixir' according to the Hindu mythology (Rawat and Roushan, 2018).

Tinospora cordifolia is an important medicinal climber used in Ayurveda, veterinary folk medicine and tribal folk medicine in various parts of the world, for treatment of various diseases and also for improving immune resistance. Recently, the plant is of great interest to researchers across the globe because of its reported medicinal benefits like anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-malarial, anti-leprotic, immunomodulatory and anti-neoplastic activities (Saha and Ghosh., 2012). Recent cytological data showed that *Tinospora cordifolia* is diploid with chromosome number 22 and basic chromosome number 11 (Jain and

Prasad, 2014). Usage of Giloy in various systems of traditional medicines of different countries has been given in Table 2.

Table 2. Usage of Giloy in different systems of traditional medicines of different countries

Sl. No.	TSM*/Country	Uses
01.	Ayurvedic	Fever, jaundice, pile, gout, hay fever
02.	Traditional Indian Medicine	Diabetes, common cold, scorpion sting (topical use), snake-bites (topical use), cancer, eye disorders, skin disorders
03.	Unani system of medicine	Anti-spasmodic, anti-inflammatory, antipyretic, chronic and seasonal fevers, anti-arthritic, anti-malarial, hepatoprotective, anti-neoplastic
05.	Japan	Fever, common cold, burning sensation, skin disorders, anti-malarial
06.	Malaysia	Diabetes, hypertension
07.	Traditional Bangladeshi medicine	Chronic diarrhoea, common cold, cough, general debility, diabetes, fever, headache, eye disorders, liver disorders, lung infections, malaria, arthritis, cancer
08.	Traditional Chinese medicine	Inflammation, fever, common cold, cough with thick sputum, gout arthritis, diarrhoea

*TSM-Traditional systems of medicine

Studies have shown that medicinal properties of the plant are due to its pharmacological attributes. The broad pharmacological effects manifested by the plant include cognition activity, anti-inflammatory and wound healing activity, anti-tuberculosis activity, hepatoprotective activity, anti-microbial activity, anti-malarial activity, anti-diabetic and cardio-protective activity, antiviral including anti-HIV & anti-SARS-CoV-2 activity (Chowdhury., 2020). These pharmacological effects are in fact due to its various constituent metabolites. Active compounds like N-methyl-2-pyrrolidone, 11-Hydroxymustakone, Magnoflorine, Tinocordiside, Syringing, N-formylannonain and Cordifolioside A obtained from plant were observed to have potential immunomodulatory and cytotoxic effects (Sharma *et al*., 2012). Different plant parts of Giloy have different pharmacological effects (Meshram *et al*. 2013), this has been summarised in Table 3.

Table 3. Medicinal uses of plant parts of Giloy (*Tinospora cordifolia*)

Sl. No.	Plant Parts	Medicinal uses
01.	Leaf	Juice or decoction of leaves is administered orally with honey in fever, ulcer and gout treatments
02.	Stem	Allays thirst, burning sensation, vomiting, enriches blood and cures jaundice
03.	Root	Roots prescribed in combination with other drugs as an anti-dote to snake bite and scorpion sting
04.	Fruit	With ghee or honey used as tonic and in treatment of jaundice and rheumatism

Giloy is one of the mandate crops of Directorate of Medicinal and Aromatic Plants Research (DMAPR), Anand, Gujarat and an elite germplasm of *Tinospora cordifolia* (INGR 06025) was developed and registered with NBPGR, New Delhi (Vision 2030, DMAPR, 2011). In Unani and Siddha systems of medicine also it occupies an important place as a main ingredient of various formulations. Extracts of Giloy plant have been used commercially in different forms such as powder, tablets, capsules etc. (Khan *et al*., 2016).

The aqueous extract of the stem has demonstrated the presence of arabinogalactan that indicated immunological activity. An arabinogalactan was also screened from the dried stems of *T. cordifolia* (Chintalwar *et al*., 2007). It is also reported that the herb contains alpha-D-glucan which is a novel polysaccharide, found to be non-cytotoxic to white blood cells (Khandagale *et al*., 2010).

Tinospora cordifolia shows great anti-cancerous activity in animal models and cell lines *in-vitro.* The anti-tumour activity of *T. cordifoli*a has been reported by various researchers but the mechanism of the action of *T. cordifolia* extract (TCE) still remains unexplained (Jagetia., 2019).

The Union Ministry of AYUSH prescribed *T. cordifoli*a as one of the prophylactic care measures against COVID-19 in its advisory- National Clinical Management Protocol based on Ayurveda and Yoga for management of COVID-19. According to the advisory, people who are at high risk of the infection or are regularly exposed to the virus are recommended to take 500 mg extract or 1-3 g powder of the

herb twice everyday with warm water for fifteen days or one month. The ministry also advises taking giloy in combination with amla (*Emblica officinalis*) and gokshura (*Tribulus terrestris*) in prescribed doses to manage symptoms like fever, sore throat, headache and tiredness (Varshney, Down To Earth, 2021).

Shree *et al*. (2020) reported that among 28 active phytochemicals from *T. cordifolia*, one compound namely Tinocordiside (CID_177384) showed highest binding affinity as compared to built-in ligand N3 for SARS-CoV-2 as per YASARA scoring. It was recorded that Tinocordiside have binding energy of 8.10 kcal/mol. Tinocordiside is found to be a new reorganized cadinane sesquiterpene glycoside from *T. cordifolia* that has the potential to inhibit the multiplication of the virus strain.

Krupanidhi *et al*. (2021) in their molecular docking study, noticed that Tinosponone, a phytochemical constituent of *T. cordifolia*, acts as a potent inhibitor of 3CL main protease of SARS-CoV-2. Molecular dynamics simulation analysis, a computer aided drug design approach was used to prove this inhibitory effect.

2.3 Status of the Crop in India and Odisha

In India giloy is extensively found throughout the country, from plains to coastal regions and hilly areas whereas, in Odisha it is commonly found in forest areas, tribal tracts, barren and waste lands. Now-a-days, it has spread to different Agro-Climatic Zones of Odisha. Recently, the plant has been listed an 'important plant' amongst the 32 prioritized plants by National Medicinal Plants Board (NMPB), New Delhi **(**Mittal *et al*., 2014). In the list of 56 prioritized medicinal plants proposed by National Medicinal Plants Board (NMPB), New Delhi, for cultivation under scheme of 30% subsidy, Giloy has been placed at 53rd position whereas, in case of Odisha it could find its place in 33 prioritized medicinal plants for commercial cultivation in the State, by State Medicinal Plants Board (SMPB) of Odisha (Swain and Kumar, 2018). Recently, Directorate of Horticulture, Government of Odisha, has identified Giloy as an emerging medicinal herb and has promoted its cultivation in all the Agro-Climatic Zones of Odisha (Swain and Kumar, 2018).

The annual consumption of the crude drug mostly by Ayurvedic pharmaceuticals/ herbal drug manufactures is estimated to be 1000t. Demand for Aqueous Giloy extract in the world market is growing due to increasing awareness and

supportive research into the use of Giloy extracts as anti-oxidant supplement, also used as supplement for improving memory, intellect, detox and blood purifier. Importers, buyers within the country, processors, traditional practitioners, Ayurvedic and Siddha drug manufacturers throng the markets for procurement of this plant every year. Its domestic as well as export demands have become quite large (Ved and Goraya 2007).

This medicinal plant has a history of getting collected indiscriminately from the wild sources without taking care of its conservation and a proper cultivation in Indian Agriculture causing a sharp decline in the availability of the drug to the industries which in turn escalated the price of the produce (Pradhan, 2016; Verma *et al*., 2019). Thus, the escalated price and sincere intervention from the government in the form of proper schemes have increased and induced the commercial interest among the farmers (Verma *et al*., 2019).

2.4 Challenges in successful production of Giloy

In recent years medicinal plants have witnessed a gradual but continuous shift from wild gathering to cultivation (Astutik *et al*., 2019). Because of growing commercial interests of farmers and constructive intervention from the government, Giloy is also in its infancy of domesticated production. Domesticated production of plants always faces some new sorts of challenges and in the changing climatic conditions emerging biotic and abiotic stresses pose a serious threat to the medicinal plants including Giloy. To cope up with this challenge "standardization of management strategies against pest and diseases and climatic variations" has been a suggested researchable issue in the Vision 2050 document of Directorate of Medicinal and Aromatic Plants Research (Vision 2050, DMAPR, 2013).

2.5 Biotic stresses in Giloy

Weeds, insect pests and diseases infesting/infecting the crop are mainly considered as the biotic stress. These constraints not only reduce the yield of the crop but also diminish the quality of the respective produce. Very few pests and diseases are reported in giloy. The plant in general is immune to most of the herbivorous pests and diseases. As far as diseases infecting Giloy and their management are concerned, very few studies have been found to address this aspect of the biotic stress.

2.5.1 Pests of Giloy plants

Hanumanthaswamy *et al*. (2010) reported that the larvae of fruit moth (*Othreis materna*) completely fed on the leaves of *T. cordifolia* during September and recorded that there was almost 20% infestation of the pest on the plant.

Gahukar (2012) observed that the adults of ash weevil (*Myllocerus viridanus* Fab.) caused notching of leaf margin in Giloy.

Marimuthu *et al*. (2018) reported that Mulberry grasshopper (*Neorthacris acuticeps acuticeps*), was found to feed on the leaves of giloy throughout the year.

2.5.2 Diseases infecting Giloy plants

In contrast to weeds and insect-pests, which mostly remain outside and affects the plant host externally, diseases being intimately associated with the host cause severe damage by reducing yield and quality. In case of medicinal plants quality is of paramount importance. To the best of our knowledge, very few attempts have been made to identify the disease affecting Giloy. Therefore, with the availability of narrow knowledge base, an attempt has been made to present a background of works done on diseases of Giloy.

Achar *et al*. (2014) reported a new leaf spot disease in *T. cordifolia* caused by *Xanthomonas campestris* in Bhadra Wildlife Sanctuary, Karnataka, India. They noticed that the disease affected almost all the above ground parts including stem, leaf lamina and midrib and occurred, particularly, during the post-monsoon months (October-November). The symptoms they observed on infected leaves were, irregular black spots with yellow halo on leaf lamina as well as on midrib, veins and in severe cases on petioles, and finally defoliation.

Shivanna *et al*. (2013) observed that *Phoma putaminum* is the causal pathogen of fungal leaf spot in *T. cordifolia*. The study indicated that the disease severity of the leaves infected with *Phoma putaminum* ranged from 0% to 100% in Bhadra Wildlife Sanctuary, Karnataka. According to their report, symptoms of disease on foliage appeared initially as chlorotic lesions that progressed to produce circular to irregular dark brown spots which enlarged and finally coalesced to develop into leaf blight,

severely infected leaves produced shot holes and extensively damaged withered off. They also reported that the alkaloid content decreased considerably due to the infection and the disease incidence and severity varied in different seasons and study regions as well as in the growing stages.

Achar *et al*. (2015) observed an interesting flat stem disease in young growing regions of *Tinospora cordifolia* during the survey for diseases in medicinal plants in Bhadra Wildlife Sanctuary, Karnataka. According to their histochemical study using Dienes' stain, the actual causal organism of the flat stem disease in *T. cordifolia* appeared to be Phytoplasma. The disease affected all the branches of infected plant, particularly, during winter season. As a result of infection, internode region became short and leaves appeared to arise from laterally extended flat rosette axis.

Mall (2015) reported the presence of foliicolous fungi, namely *Colletotrichum capsici* and *Pseudocercospora cocculi* on *Tinospora* sp. from North Central Terai Forests of Uttar Pradesh, India.

2.6 Diseases in medicinal plants caused by *Alternaria alternata*

Kamalakannan *et al*. (2007) reported that *Alternaria alternata* caused leaf spot of *Aloe barabadensis.*

Patil *et al*. (2009) reported that the leaf spot disease of *Withania somnifera* was caused by *Alternaria alternata*.

Maiti *et al*. (2007) detailed on the association of *Alternaria alternata* with leaf spot of *Aloe vera* and leaf blight of *Rauvolfia serpentina*, *Mentha arvensis*, *Ocimum gratissimum*, *Plantago ovata*, *Catharanthus roseus*, *Cassia angustifolia* and *Datura metel.*

MATERIALS AND METHODS

The investigation entitled **"Symtomatology and etiology of leaf spot disease of giloy (*Tinospora cordifolia* Thunb.)"** was carried out by performing a comprehensive survey, in the experimental farm of All India Coordinated Research Project on Medicinal & Aromatic Plants and Betelvine located at a Global grid of 20° 16' N Latitude and 85° 47' E Longitude with an elevation of 45 m above the sea level, at Horticultural Research Station, Baramunda Farm, of Odisha University of Agriculture and Technology (OUAT), Bhubaneswar. Also, the survey was extended to Jatni (Khordha), Pipili (Puri), Balipatna (Khordha) and Cuttack regions of Odisha, from where diseased samples were collected for studies. Laboratory works were conducted in the Laboratory of Department of Plant Pathology, College of Agriculture, OUAT, Bhubaneswar. Odisha during the year 2020-21. The details of the materials used and methods employed in carrying out the present research are mentioned in this chapter.

3.1 Materials

3.1.1 Diseased samples

The diseased leaf samples of giloy showing leaf spot symptoms were collected from five different locations, near Bhubaneswar and brought to the laboratory for further studies. Locations from where diseased samples were gathered are mentioned below:

Sl. No	Place of Collection	District
1.	AICRP on MAP & Betelvine, OUAT, Bhubaneswar	Khordha
2.	Jatni	Khordha
3.	Cuttack	Cuttack
4.	Balipatna	Khordha
5.	Pipili	Puri

3.1.2 Glasswares

The standard Borosil brand glass wares such as Petri plates, test tubes, conical flasks, measuring cylinder, funnel, glass rods, slides, cover slips, spirit jar etc., were used during the course of investigation. They were kept submerged overnight in the cleaning solution prepared by dissolving 60 g Potassium dichromate ($K_2Cr_2O_7$) and 60 ml of concentrated Sulphuric acid (H_2SO4) in 1 L of water. Later, these glasswares were cleaned by washing with detergent solution followed by rinsing with tap water and distilled water.

3.1.3 Surface disinfectants

Ethyl alcohol (70%) was used for disinfecting Laminar Air Flow chamber before commencing inoculation procedures and Sodium hypochlorite (NaOCl) 1.0% solution was used for surface sterilization of plant samples in the laboratory.

3.1.4 Culture media

Potato dextrose agar (PDA) medium and Nutrient Agar (NA) medium in separate Petri plates were used initially to place diseased samples. Later, for isolating, culturing and sub-culturing the microorganisms PDA was used. For further nutritional studies of the pathogen, in addition to Potato Dextrose Agar, other culture media viz., Oat meal agar, Ashby's agar, Czapek's dox agar and Richard's agar were used.

PDA medium was prepared in the laboratory using commercially available potato from local market and dextrose and agar-agar from HIMEDIA Pvt. Ltd (Mumbai, India). Nutrient Agar, Czapek's dox Agar, Richard's Agar, Oat meal Agar and Ashby's agar used for cultural studies of the pathogen was also from HIMEDIA Pvt. Ltd (Mumbai, India). Composition of different media used in the laboratory is mentioned in Table 4.

Table 4: Composition of different culture media

Sl. No	Name of the Media	Ingredients	g/L
1.	Potato Dextrose Agar	Peeled potato Dextrose Agar agar Distilled water	200 20 20 1000 ml
2.	Nutrient Agar	Peptone Sodium chloride HM Peptone B Yeast Extract Agar Distilled water	5 5 1.5 1.5 15 1000 ml
3.	Czapek's dox Agar	Sucrose Sodium nitrate Dipotassium phosphate Magnesium sulphate Potassium chloride Ferrous sulphate Agar agar Distilled water	30 2 1 0.5 0.5 0.01 15 1000 ml
4.	Richard's Agar	Potassium nitrate Potassium dihydrogen phosphate Magnesium sulphate Ferric chloride Potato starch Sucrose Agar agar Distilled water	10 5 0.25 0.02 10 50 20 1000 ml
5.	Oat meal Agar	Powdered oat meal Agar agar Distilled water	30 20 1000 ml
6.	Ashby's agar	Mannitol agar Potassium diphosphate Magnesium sulphate Sodium chloride Potassium sulphate Calcium carbonate Agar agar Distilled water	20 0.20 0.20 0.20 0.10 5.0 15 1000 ml
Final pH (at 25ºC) 7.0 $\pm$ 0.2			

3.1.5 Equipments

The standard laboratory equipments such as autoclave, BOD incubator, laminar air flow, hot air oven, refrigerator, electronic weighing balance, research microscope, stereoscopic binocular microscope, water distillation unit, ocular micrometer, stage micrometer, pH meters etc. were used during the experimental work.

3.1.6 Other Materials used in Laboratory and Field

This include, inoculating needles, forceps, spirit lamp, cotton, sodium hypochlorite, cork borer, blotter papers, non-absorbent cotton, labels, polythene bags, permanent marker etc., that were used during the present investigation.

3.2 Methods

3.2.1 Observation of diseases

Giloy plants growing in the experimental plots and herbal garden of AICRP on MAP and Betelvine, Jatni, Balipatna, Pipili and Cuttack regions of Odisha were randomly observed at regular intervals to check the onset of leaf spot diseases in the plants. Severity of leaf spot diseases in *Tinospora cordifolia*, in per cent was calculated using the following formula:

$$\text{Disease severity index (\%)} = \frac{\text{Sum of all ratings}}{\text{Total ratings x Maximum disease grade}} \text{ x } 100$$

For calculating disease severity, ten diseased Giloy plants were randomly selected from each location and then five to ten diseased leaves, depending upon the infection, were chosen from each selected plant. Based upon the per cent of the leaf area affected by the disease, the chosen leaves were assigned a numerical rating following 1-9 numerical rating scale as per the guidelines of National Innovations in Climate Resilient Agriculture (NICRA) for foliar diseases, 2011. The details of numerical rating scale are mentioned below in Table 5.

Table 5. Disease scale for calculating disease severity.

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

3.2.2 Collection of diseased samples

Giloy leaves with leaf spot symptoms were collected from five different localities near Bhubaneswar, Odisha, in separate sterile polythene bags, to avoid contamination and were brought to the laboratory. Three samples were collected from each location. The polythene bags were labelled and stored at 4°C until isolation of the pathogen.

3.2.3 Description of symptoms

Symptoms observed on diseased plant samples were carefully examined and recorded. Emphasis was given to the shape, size and colour of the leaf spot for their systematic description.

3.2.4 Sterilization of glass wares and other materials

The glasswares were properly washed with detergent powder and dried in air. Thereafter, they were wrapped in clean brown paper and sterilized in hot air oven at 180^{o}C temperature for 1 hr. Autoclave was used to sterilize culture medium and distilled water at 1.054 kg/Sq. cm pressure for 20 minutes.

3.2.5 Isolation of pathogen

The fresh samples showing leaf spot symptoms were brought to the laboratory. These samples were washed in tap water to remove dirt and then dried in air. The affected samples were then cut into small pieces with a sterilized scalpel. The small

pieces were then surface sterilized in sodium hypochlorite (1 %) for 1½ to 2 minutes, washed in three changes of sterilized water to remove traces of sodium hypochlorite (NaOCl) and were then dried on sterilized blotting paper.

These small pieces were then aseptically placed on previously sterilized PDA medium and NA medium poured in Petri plates under aseptic conditions with the help of flamed forceps. The plates were then incubated at 27 ± 10ºC and were observed periodically for the growth of pathogen, till any visible bacterial colony/hyphal mass appeared around the pieces.

3.2.6 Purification of the isolated organisms

The isolated pathogen, if fungus, can be purified by hyphal tip method. In this method, tip of hyphae from a germinating mycelium is carefully lifted up along with a small bit of medium with the help of a sterilised inoculating needle. This is then aseptically transferred to PDA slants and kept for incubation at room temperature.

The isolated pathogen, if bacteria, can be purified by streak plate method. In this method, the bacteria are spread on the surface of a solid medium aseptically, using an inoculation loop, so that single cell occupies an isolated portion of the agar surface.

3.2.7 Maintenance of culture

Pathogen cultures are sub-cultured on slants of suitable media and incubated at temperature of 27 ± 10ºC for 10 days. Such slants were preserved in a refrigerator at 5-10ºC. These cultures were sub-cultured once in a month to maintain viability of the pathogen.

3.2.8 Identification of the pathogen

Pathogen was studied under microscope. Attempt was made to observe fungal structures, fruiting bodies, spores, conidia or bacterial cells. Further, identification of the pathogen was done based on cultural and morphological characteristics.

3.2.9 Pathogenicity test

For pathogenicity test, healthy Giloy plants (21 days old) grown in polythene bags (4'' x7”), without any spots or blemishes are selected and their leaves are washed properly. Later, these leaves can be inoculated with pathogenic suspension of 7-10 days old culture grown on suitable media/broth. This suspension is sprayed using a hand

atomizer on the leaves of the Giloy plants, on which prior injuries are made with the help of a sterilised needle. The inoculated plants are kept at high humidity for 48 hrs, after which the polybags are transferred to cage house. Observations for development of symptoms are recorded 7 days after inoculation.

3.2.10 Re-isolation

The leaf spots produced by the inoculated pathogen are subjected to re-isolation procedures. The re-isolation is made on suitable medium. The cultures obtained from inoculated plant leaves are compared with those of original cultures, to prove the pathogenicity of the causal organism.

3.3 Morphological characters

The morphological characters of the pathogen infecting Giloy and initiating leaf spot disease, is studied and recorded. Observations regarding different morphological structures are made by adopting slide culture technique. The microscopic measurements are taken with the help of a micrometer. Averages based on 20 observations for each structure, recorded from 5 different slides of 10 randomly selected individuals from each slide are considered.

3.4 Cultural characters

The pathogen isolated from leaf spots are grown on different media mentioned in section 3.1.4 of the chapter Materials and Methods, to study the growth and other required cultural characters respectively. Approximately 20 ml of suitable media is poured into fifteen Petri plates of 90 mm diameter. Media in these plates are allowed to solidify and then inoculated with the fungal/bacterial pathogen using a sterile cork borer/ inoculation loop. These plates are then incubated at 27 $\pm$ 1ºC for five to seven days.

EXPERIMENTAL RESULTS

Present study was carried out in three major aspects. Firstly, collection and description of leaf spot disease affecting Giloy plants grown in the herbal garden of AICRP on MAP & Betelvine, OUAT, Bhubaneswar and different regions near to Bhubaneswar, Odisha. Importance was also given to progress of symptoms and signs, if any, of the disease. Secondly, carrying out pathogenicity test to prove the pathogenicity of isolated pathogen. Thirdly, morphological and cultural characterization of causal organism and its identification. The detailed results of the investigation are mentioned below under the following sub-headings.

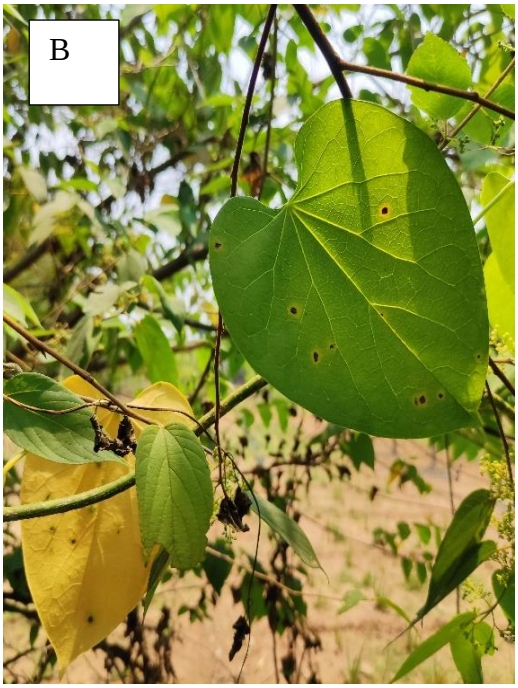
4.1 Collection of diseased samples and description of leaf spot symptom

4.1.1 Collection of diseased samples

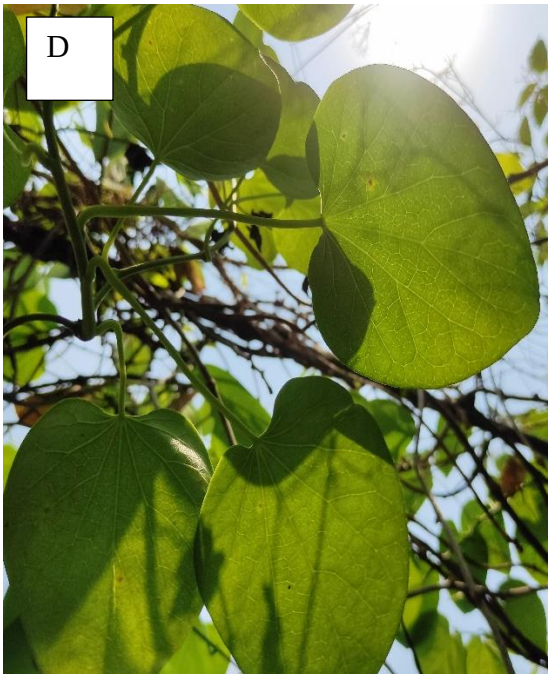
Giloy plants growing in experimental plots and herbal garden of All India Coordinated Research Project on Medicinal and Aromatic Plants & Betelvine, OUAT, Bhubaneswar, was observed regularly for disease occurrence. Leaf spot disease was noticed on both young and mature giloy plants. Diseased leaf samples were also collected from Jatni (Khordha), Pipili (Puri), Balipatna (Khordha) and Cuttack regions of Odisha to know the severity of leaf spot disease occuring in these regions (Fig 4.1). Three samples were collected randomly from each location. Disease severity rating was assigned for leaf spot disease occurring in different places surveyed, this was based on numerical rating following 1-9 numerical rating scale (as per the guideline of NICRA for foliar diseases, 2011). The details of numerical rating scale are mentioned in Table 5. For calculating disease severity, ten diseased Giloy plants were randomly selected from each location and then five to ten diseased leaves, were chosen from each selected plant, depending upon the infection rate.

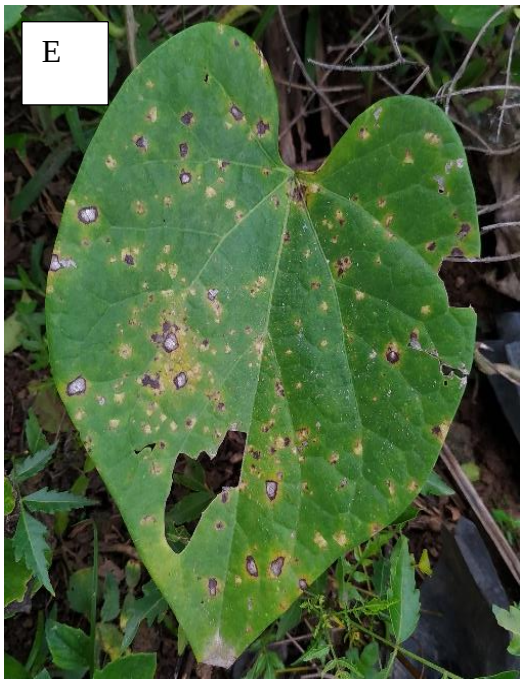
The symptoms observed on the diseased samples collected has been recorded and abridged in Table 6.


A


B


C





A- AICRP on MAP and Betelvine, Bhubaneswar **B- Cuttack**

C- Jatni **D- Pipili**

E- Balipatna

Fig 4.1: Variation in disease symptoms as per location

Table 6. Symptoms of leaf spot disease on collected samples

Sl. No	Samples/ Isolates	Place of collection	Average Disease severity	Symptoms observed
1.	TC 1 A	AICRP on MAP and Betelvine, Bhubaneswar	8	Circular to irregular dark brown spots with concentric rings and yellow halo were observed.
	TC 1 B			
	TC 1 C			
2.	TC 2 A	Jatni	6	Water-soaked irregular necrotic lesions with yellow to dark brown margins.
	TC 2 B			
	TC 2 C			
3.	TC 3 A	Pipili	3	Small dot like spots with light brown colour observed on ventral surface of leaves, corresponding dorsal side were yellow in colour.
	TC 3 B			
	TC 3 C			
4.	TC 4 A	Balipatna	5	Grey to white centered larger spots with distinct yellow to dark brown margins observed on leaves.
	TC 4 B			
	TC 4 C			
5.	TC 5 A	Cuttack	7	Larger spots with membranous papery center observed. Entire leaf turned yellow in colour. Shot holes symptoms were common.
	TC 5 B			
	TC 5 C			

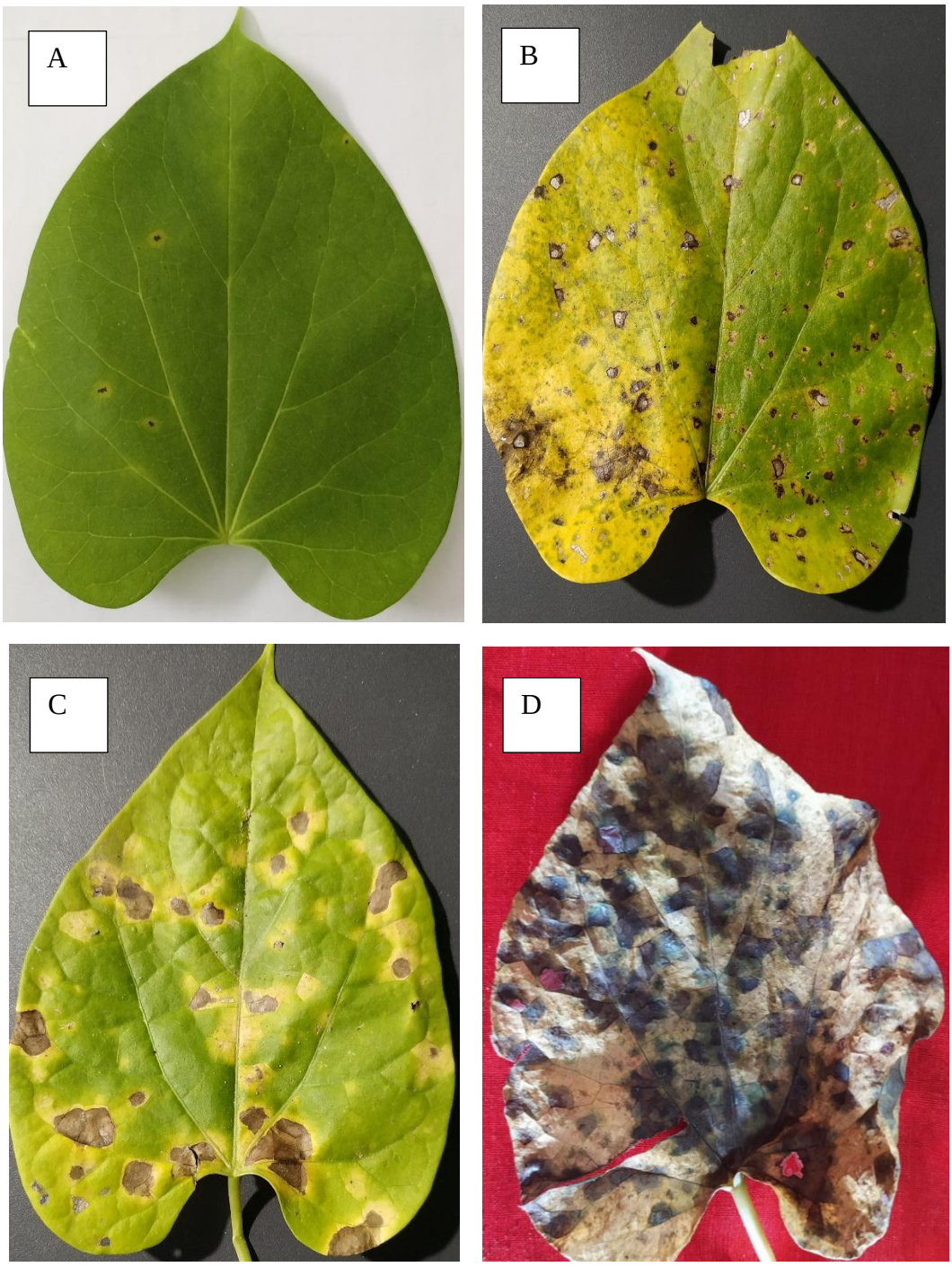
4.1.2 Description of symptoms

The spots were prominently noticed on leaves compared to other above ground parts of the plant. The leaf spots in Giloy initiated with small water-soaked lesions turning into round, light brown coloured dot-like spots (Fig 4.2. A). These dot-like spots increased in size to become larger circular to oval, dark brown to greyish spots with distinct yellow border initially and dark brown margin later (Fig. 4.2. B).

Spots were more prominent on the ventral side of leaves compared to their dorsal side. Later, the spots enlarged into round to irregular lesions with concentric rings (Fig.4.2. C). The diameter of the spots measured from 2mm to 5 mm in some blighted leaves. The spots were surrounded by chlorotic zone with greyish white necrotic centers. As the disease progressed, the greyish center became brittle and papery leading to creation of shot holes. In severe condition, the entire leaf became blighted, causing withering and defoliation of leaves (Fig. 4.2. D).

Isolates collected from AICRP on MAP and Betelvine, Bhubaneswar (TC 1A, TC 1B, TC 1C) showed circular to irregular dark brown spots with concentric rings and yellow halo on leaves. These symptoms were noticed in almost all leaves of selected plants in the place. Water-soaked irregular necrotic lesions with yellow to dark brown margins on leaves were seen in isolates obtained from Jatni region (TC 2A, TC 2B, TC 3C). Leaf samples collected from Pipili (TC 3A, TC 3B, TC 3C) appeared to have grey to white centered larger spots with distinct yellow to dark brown margins. Grey to white centered larger spots with distinct yellow to dark brown margins appeared on leaves samples collected from Balipatna (TC 4A, TC 4B, TC 4C), whereas isolates obtained from Cuttack region (TC 5A, TC 5B, TC 5C) manifested larger spots with membranous papery center. Many times, entire leaf turned yellow in colour and shot holes symptoms were common.

Disease was found to be prevalent in all the surveyed localities, but disease severity varied between 3 to 8. The disease severity was maximum at herbal garden of All India Coordinated Research Project on Medicinal and Aromatic Plants and Betelvine, OUAT, Bhubaneswar, with a disease severity rating of 8, followed by Cuttack and Jatni, which had a disease severity grade of 7 and 6 respectively. Disease severity in Balipatna was graded as 5, while Pipili had the lowest disease severity with a rating of 3.



A- Initial symptoms **B- Spots with distinct margins**

C-Irregular lesions **D- Blighted leaves.**

Fig 4.2: Progress of leaf spot symptoms on Giloy leaves

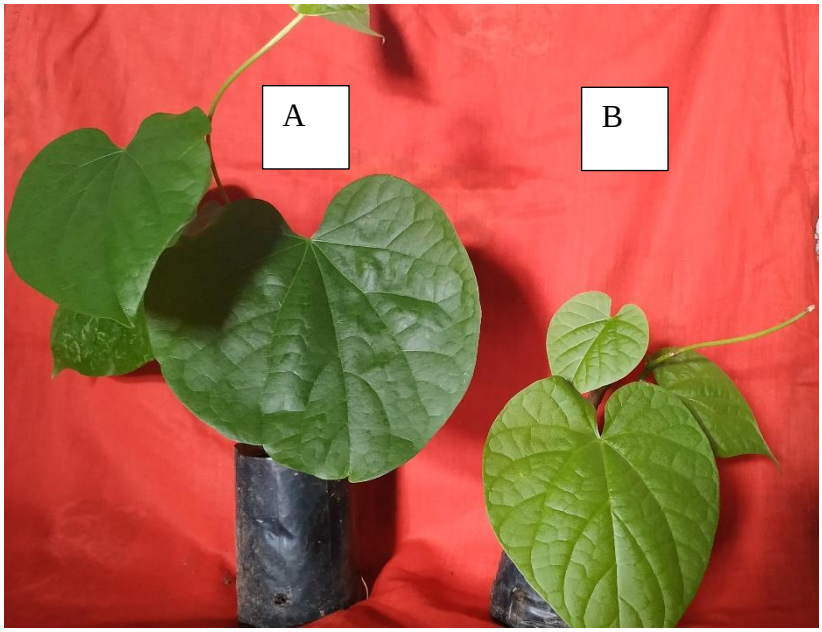
4.2 Pathogenicity test

4.2.1 Pathogenicity of isolates

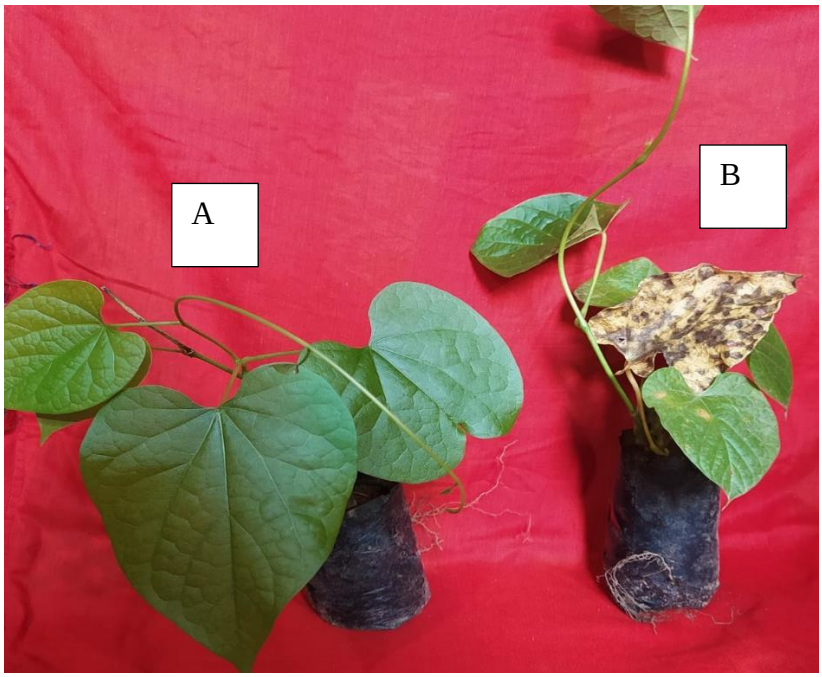
For pathogenicity test, healthy Giloy plants (21 days old) grown in polythene bags (4'' x7"), without any spots or blemishes were selected and their leaves were washed properly. Later, these leaves were inoculated with mycelial suspension of different fungal pathogen of 7 days old culture grown on potato dextrose broth which was filtered and homogenized to give $1x10^3$ viable propagules per ml. This suspension was sprayed using a hand atomizer on the leaves of the Giloy plants, on which prior injuries were made with the help of a sterilised needle.

The inoculated plants were kept at high humidity for 48 hrs, after which the polybags were transferred to cage house. Observations for development of symptoms were recorded after inoculation (Fig.4.3). Pathogenicity of different isolates inoculated on Giloy plants were recorded and is summarized in Table 7.

From the data from Table 7. it is clear that minimum days required for initiation of symptoms after artificial inoculation of healthy Giloy plants were 3 days for most of the isolates, while for complete expression of leaf spot on plants the pathogen required in most cases a minimum of 8 days. Symptoms produced on the plant by the pathogen were similar in all cases considered.



I. Before Pathogenicity Test



II. After Pathogenicity test

A-Healthy Giloy Plants **B-Inoculated Giloy Plants**

Fig 4.3: Pathogenicity test

Table 7. Pathogenicity of isolated organisms

Sl. No	Isolates	Days required for initiation of symptoms	Days required for complete expression of symptoms	Observations
1.	TC 1A	2	8	Initially circular spots but later enlarged into irregular dark brown lesions with concentric rings and white to greyish center. The spots were surrounded with yellow margin and subsequently turned dark brown. On progress of the disease, spots coalesced to form larger blighted regions and the extensively damaged leaves defoliated.
2.	TC 2A	3	8	
3.	TC 2C	3	7	
4.	TC 3B	3	7	
5.	TC 3C	3	8	
6.	TC 4B	4	8	
7.	TC 4C	2	8	
8.	TC 5A	4	8	
9.	TC 5B	3	8	
10.	TC 1B	Nil	Nil	No development of symptoms
11.	TC 1C			
12.	TC 2B			
13.	TC 3A			
14.	TC 4A			
15.	TC 5C			

On carrying out pathogenicity test, it was observed that isolates TC 1A, TC 2A, TC 2C, TC 3B, TC 3C, TC 4B, TC 4C, TC 5A and TC 5B were pathogenic on Giloy plants. The plants inoculated with these isolates manifested leaf spot symptoms similar to that present under natural conditions. Affected/inoculated leaves showed initially dot like circular spots that later enlarged into irregular dark brown lesions with concentric rings and white to greyish center. The spots were surrounded with yellow margin that subsequently turned dark brown. On progress of the disease, spots coalesced to form larger blighted regions and the extensively damaged leaves withered off. The pathogen was re-isolated from the infected plant parts and purified on PDA medium. The diseased spots yielded the greyish black fluffy and raised fungus culture, identical to original fungus used for inoculation.

The isolates TC 1B, TC 1C, TC 2B, TC 3A, TC 4A, TC 5C when inoculated on healthy Giloy plants failed to initiate disease symptoms even after 7 days. Hence, it was concluded that they were saprophytes and not the causal organism of leaf spot disease in Giloy and was discarded from further studies.

4.3 Characterization of fungus isolated from Leaf spot disease of Giloy and its Identification

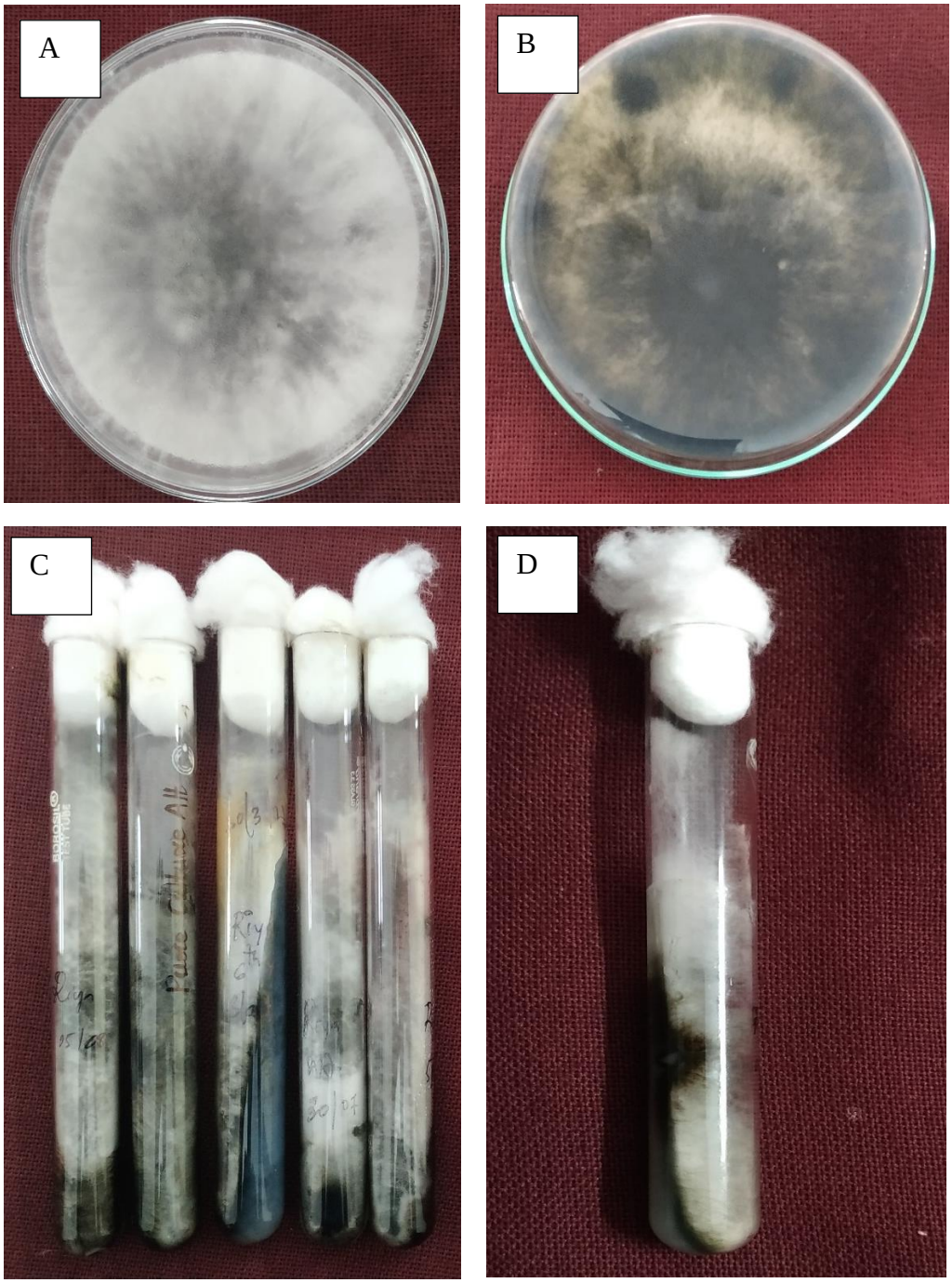
4.3.1 Cultural studies

Symptomatically, it appeared that the disease could be caused by a fungal or bacterial agent and thus, attempt was made to isolate the associated pathogen on PDA medium or NA medium by tissue isolation technique as mentioned in the Chapter on Materials and Methods. The samples collected from different places were surface sterilized and placed in both PDA and NA media under aseptic conditions to study various cultural characters exhibited by the fungus. Different observations recorded are summarized in the Table 8.

Table 8. Cultural characteristics of different isolates

Sl. No	Sample/ Isolate No.	Observations recorded			
		Growth on PDA		Growth on NA	
		3rd day	7th day	3rd day	7th day
1.	TC 1A	Small white fluffy mycelial growth observed.	Greyish black, mycelia seen,	No evident growth.	Very small mycelial fragments observed
2.	TC 2A	Creamy white larger hyphal mass was seen.	Abundant Greyish black mycelia with even white margins observed on the media.	Small white mycelial fragments seen.	No evident change.
3.	TC 2C	Black spores on media.	Dark brown to black spores covered entire surface of media.	No evident growth.	No evident growth.
4.	TC 3B	Off white smaller hyphal colony seen.	Mycelium turned fluffy and dark brown to greyish black in colour.	No evident growth.	No evident growth.

5.	TC 3C	Small white fluffy mycelial growth observed.	Greyish black, raised fluffy aerial mycelia seen,	No evident growth.	No evident growth.
6.	TC 4B	Creamy white to light grey hyphal mass was seen.	Abundant Greyish black mycelia with even margins observed on the media.	No evident growth.	No evident growth.
7.	TC 4C	Light grey hyphal colony observed.	Greyish black to dark brown raised mycelial growth.	No evident growth.	No evident growth.
8.	TC 5A	Cream to grey hyphal colony observed.	Greyish black fluffy mycelial growth observed on the media. The mycelia covered the entire diameter of the Petri plate.	No evident growth.	Dark black mycelial fragments seen.
9.	TC 5B	Cream to light grey hyphal colony observed.	Greyish black to dark brown raised and fluffy mycelial growth seen.	No evident growth.	Olive green and black intermingled mycelium on media



A- Front side of Petri plate **B-Reverse side of Petri plates**

C, D-Pure culture of fungus in PDA slants

Fig 4.4: Pure culture of fungus in Petri plates and Slants

From the observations recorded, the causal agent of leaf spot disease appeared to be a fungus due to amplified growth of fungal mycelium on PDA and small mycelial fragments on NA medium. The proliferated greyish black fungal mycelium was observed on many incubated plates with PDA medium after 7 days. Also, in some plates (TC 2C) black spores covered the entire surface of the media. These mycelia were sub cultured on PDA medium to obtain pure cultures.

Isolates from each locality, with good mycelial growth on PDA (TC 1A, TC 2A, TC 3B, TC 3C, TC 4B, TC 4C, TC 5A, TC 5B) was maintained for further studies. The culture tubes and the Petri plates were maintained at lower temperature (4°C) in a refrigerator (Fig.4.4). Sub-culturing was repeated at an interval of 15-20 days.

4.3.2 Morphological studies

Morphological observations of the fungus were recorded by adopting slide culture technique. The fungus produced profuse mycelial growth on PDA. The isolated fungus was characterized based on its macro morphology (colony characteristics) and micro morphology (description of structures observed under microscope). Initially, the mycelium was off white/cream in colour, fluffy and raised with plenty of aerial mycelium when cultured in PDA medium. Later, the fungal colony turned to dark greyish black colour which can be very well observed from the reverse side of the Petri plate (Fig. 4.4 B).

In case of micro morphology, observation on shape, size, colour etc., of hyphae/mycelium, conidia and conidiophore of isolated fungus was made under microscope. The different morphological features of the fungus observed under microscope are presented in Table 9.

1. Mycelium

Hyphae observed was filamentous, hyaline, multi-celled, septate, irregularly branched, and thin (2.94 µm in diameter) initially but became slightly thick (4.5 µm) and light grey in colour as they grew old (Fig.4.5 A).

2. Conidiophore

The conidiophores were straight, erect, septate, golden brown in colour, measuring 42.32 µm (27.35-110 µm) in length and 5.12 µm (3.32-7.43 µm) in width. The length : width ratio of conidiophore was 8.26 µm (8.23-14.80 µm).

3. Conidia

Conidia were born in chains on conidiophores. They were golden to light brown in colour and varied in shape from obclavate to mostly muriform, tapering gradually towards apex with 2-3 transverse septa and usually several longitudinal septa. (Fig. 4.5 B). The muriform conidia inclusive of beak measured 45.23 µm (25.82-102.57 µm) x 14.67 µm (9.45-18.89 µm). Length of the conidium was 2-5 times more than its width.

Table 9: Morphological characters of the fungus.

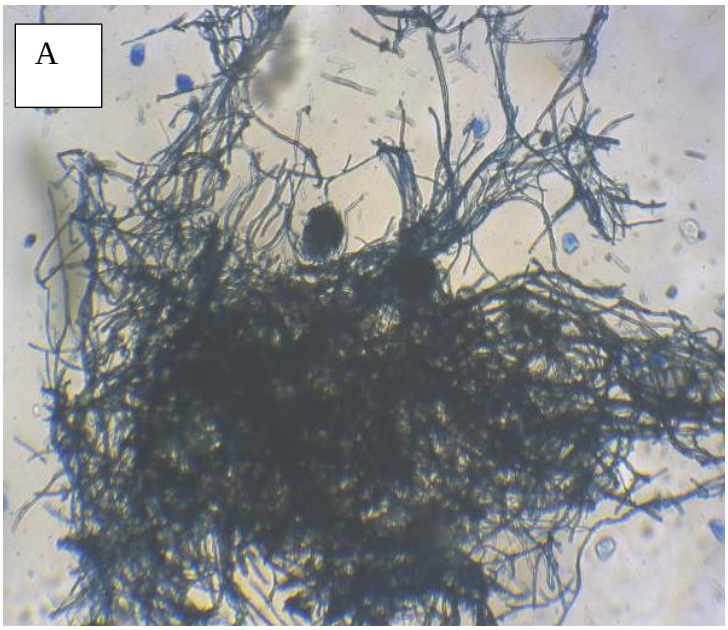
Structures	Morphological characters			
	Shape	**Size***	**Colour**	**Septation**
Mycelium	Filamentous	4.50 µm in width	Hyaline to light grey	Septate
Conidia (inclusive of beak)	Obclavate to muriform	45.23 x 14.67 µm	Golden to light brown	2-3 transverse septate and several longitudinal septa
Conidiophores	Straight Erect	42.32 x 5.12 µm	Golden brown	Septate

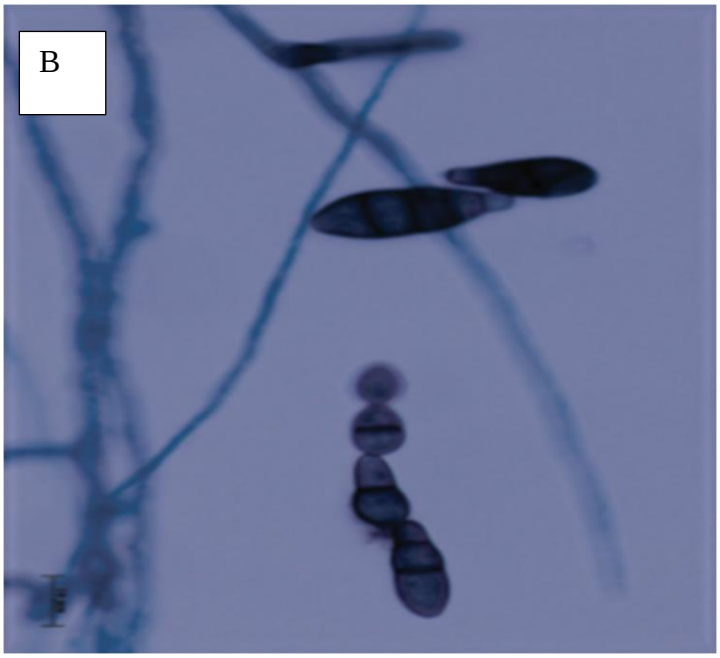
*Averages based on 20 observations of 5 randomly selected isolates.

4.3.3 Identification of fungus isolated from Leaf spot disease of Giloy

The fungi studied, produced greyish black fluffy mycelial colonies on PDA. It was filamentous, hyaline to light grey in colour, multi-celled, septate, irregularly branched and had non-septate, beaked, golden brown muriform conidia borne on straight and erect light brown conidiophore.

Based on the above characters of the fungus, reference materials and expert opinion of mycologists from Department of Plant Pathology, OUAT, the fungus was identified as ***Alternaria alternata***. The isolates TC 1A, TC 2A, TC 3B, TC 3C, TC 4B, TC 4C, TC 5A and TC 5B were all same.





A- Mycelium of fungus under 10X magnification
B-Muriform conidia of fungus observed under high magnification.

Fig 4.5: Mycelium and conidia of fungus under microscope

4.4. Nutritional studies

For conducting nutritional studies, five different media were used viz., Potato Dextrose Agar, Czapek's dox Agar, Richard's Agar, Oat Meal Agar and Ashby's Agar. Composition of these media are mentioned in Table 4 of chapter Materials and Methods. Pathogen exhibited varying degrees of growth and sporulation on different media (Table 11).

1. Growth characters

The colony diameter of the pathogen varied as per culture media (Table 10). The mean colony diameter was calculated taking into account, average colony growth in five replications of each media. The media *viz*., Potato dextrose agar and Richard's agar showed significantly maximum mean colony diameter of 90 mm and 83 mm respectively. It was followed by Oat meal agar and Czapek's dox agar which recorded 75 mm and 62 mm mean colony diameter. On the contrary, least mean colony diameter was observed on Ashby's agar (51 mm).

In PDA, circular fungal colonies with even margins and greyish to black colour fluffy aerial mycelia was observed while, in Czapek's dox Agar, thick profusely grown light olive colour mycelial colonies with circular margin were noticed. Flat and circular colonies with poor dark olive-green mycelial growth was observed on Oat Meal Agar. On Ashby's Agar, colonies with circular margin and profuse aerial mycelium were noticed with light grey center whereas, on Richard's Agar, light and dark greyish mycelium showed profuse growth and the colonies had irregular margins.

Table 10. Radial growth of pathogen on different culture media

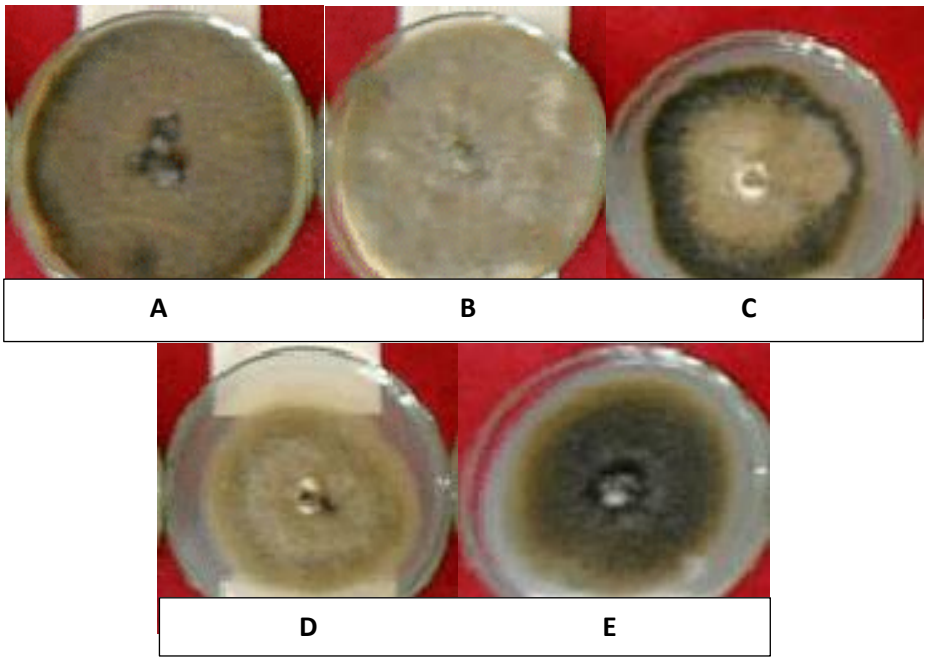
Sl. No	Media	Radial growth of fungus (mm)					Average (mm)
		R1	R2	R3	R4	R5	
1.	Potato Dextrose Agar	88	95.8	89	84.2	93	90
2	Czapek's dox Agar	64.6	60	58	63.4	64	62
3.	Oat Meal Agar	78	74.1	71.9	78.7	72.3	75
4.	Ashby's Agar	48	55	52.5	49.5	50	51
5.	Richard's Agar	80	87.2	82.5	80	85.3	83
	S.Em ±	1.5036					
	CD at 5%	4.4349					
	C.V %	0.9313					

2. Sporulation

With regard to sporulation, abundant growth and sporulation was noticed on Potato dextrose agar and Richard's agar, whereas good sporulation was observed on Oat meal agar and moderate sporulation was observed on Czapek's dox agar and Ashby's agar. Sporulation of fungal pathogen on different media is summarized in Table 11.

Thus, based on the nutritional studies conducted, it was observed that Potato Dextrose Agar best supported the mycelial growth of the fungus, on which the fungus attained a mean colony diameter of 90 mm along with abundant sporulation. This was followed by Richard's Agar, on which the pathogen grew upto 83 mm in diameter and sporulated abundantly. It was noted that Ashby's Agar was least suitable for the

pathogen as it showed lowest colony diameter and moderate sporulation on the media (Fig.4.6). Observations recorded are presented in Table 11.



A-Potato Dextrose Agar

B-Richard's Agar

C-Oat Meal Agar

D- Czapek's dox Agar

E-Ashby's Agar

Fig.4.6: Mycelial growth of *Alternaria alternata* on different solid media.

Table 11. Radial growth and sporulation of A. alternata on different culture media.

Sl. No	Name of the Media	Mean Colony Diameter (mm) *	Sporulation	Growth Characters
1.	Potato Dextrose Agar	90	++++	Colonies circular, even margin, mostly greyish in colour, margin is dull white with profuse growth
2.	Czapek's dox Agar	62	++	Colonies with circular margin, thick mycelium profusely grown with light olive in colour
3.	Oat meal agar	75	+++	Colonies flat and circular, poor mycelial growth and colour is dark olive green with olive grey at periphery
4.	Ashby's agar	51	++	Colonies with circular margin, aerial mycelium profusely grown at the centre with light grey in colour
5.	Richard's Agar	83	++++	Colonies with irregular margin, profusely grown mycelium and light and dark greyish in colour

* Average of five replications; + = Scanty sporulation, ++ = Moderate sporulation, +++ = Good sporulation, ++++ = Abundant sporulation

DISCUSSION

Tinospora cordifolia is one of the highly valued herbs in Indian medicine. It is commonly known as heart-leaved moonseed, guduchi or giloy. It finds its application in the treatment of cancer, asthma, diabetes, blood pressure, skin diseases, allergic conditions and of late in the treatment of novel Coronavirus. Besides these, it is also used as an appetizer, febrifuge, stomachic, cardiotonic, antihelminthic, and adaptogen. *Tinospora cordifolia* grows extensively throughout India and invariably it has been observed that the plant is affected by many foliar diseases which result in premature defoliation and death of the plant. This leads to reduction in yield of the shrub and affects the quality of the bioactive compounds extracted from different plant parts.

Achar *et al.* in 2014 and 2015 reported foliar diseases caused by *Xanthomonas campestris* and Phytoplasma in Giloy plants growing in Bhadra Wildlife Sanctuary in Western Ghats region of Karnataka. Shivanna *et al.* in 2013 reported fungus *Phoma putaminum* causing diseases in Giloy. The diseases caused by these organisms produced various symptoms such as, small yellow spots turning into dark brown to black lesions surrounded by yellow halo. They developed a necrotic center and finally cell death was observed. These conditions have been favoured by prolonged wet and humid conditions which are helpful in dissemination of the pathogens involved. Barring these few diseases, not much of work has been done on diseases of Giloy and their causal agents.

The present study was attempted to describe one of the foliar diseases predominantly occurring in and around Bhubaneswar, Odisha. Leaf samples showing presence of leaf spots were collected and their symptoms described. The spots initiated with water-soaked lesions which turned to round light brown colour dot like spots. Overtime, these spots increased in size to become larger, circular to oval, dark brown to greyish spots with distinct yellow border. The spots were more on the ventral side and at the later stage these spots enlarged to round irregular lesions with concentric rings. The diameter of spots measured from 2mm to 5mm. The spots were surrounded by chlorotic zone with greyish-white necrotic centers. As the disease progressed the greyish center became brittle and papery leading to formation of shot holes. In severe conditions the entire leaf become blighted, withers and get defoliated. Samples from all the places of collection were more or less similar with little variations.

Achar *et al.* in 2014 and 2015 have reported foliar diseases in Giloy but did not describe the symptoms in detail. They made a mention of the chlorotic lesions that progressed to produce circular to irregular dark brown spots which enlarged and finally coalesced to develop into leaf blight. Under severe conditions, shot holes were formed.

The present description of symptoms of the leaf spot disease encountered does not seem to have any similarity with that described by Achar *et al*. hence, regarded as a new encounter.

Pathogenicity test of isolates from leaf samples collected from different places were done and out of 15 isolates only 9 isolates could produce the symptoms that were observed on the original sample. The rest isolates did not produce any symptoms and were regarded as saprophytes and thus, excluded from further studies.

Cultural and morphological characterisation of the virulent isolates was done. On Potato Dextrose Agar culture plates, all the isolates had white fluffy aerial mycelial growth initially with little variations like creamy and light olivaceous grey colouration in some cases. At later stage, the mycelial colour changed to greyish black. In one case (TC 2C) black spores covered the entire media surface.

The microscopic studies revealed that the hyphae were filamentous, hyaline, multi-celled, septate, irregularly branched, and thin (2.94 µm in diameter) initially but became slightly thick (4.5 µm in diameter) and light grey in colour as they grew old. The conidiophores were straight, erect, septate, golden brown in colour, measuring 42.32 µm in length and 5.12 µm in width. The conidia were born in chains on conidiophores. They were golden to light brown in colour and varied in shape from obclavate to mostly muriform, tapering gradually towards apex with 2-3 transverse septa and usually several longitudinal septa.

There are only few workers who studied diseases of Giloy but have not described the pathogen associated, hence, the present attempt to describe the pathogen associated is a systematic attempt to identify the pathogen.

Based on the cultural and morphological characterisation, the fungus was identified as ***Alternaria alternata*** with the help of mycologists from the Department of Plant Pathology, Orissa University of Agriculture and Technology, Bhubaneswar. No earlier worker has reported the infection of *Tinospora cordifolia* by *Alternaria*

alternata. However, the pathogen has been reported from other medicinal plants like *Aloe barabadensis, Withania somnifera, Rauvolfia serpentina, Mentha arvensis, Ocimum gratissimum, Plantago ovata, Catharanthus roseus, Cassia angustifolia* and *Datura metel* by Kamalakannan *et al*. (2007)., Patil *et al*. (2007) and Maiti *et al*. (2007). So, this finding is novel to the literature.

An effort was also made to find out the best source of nutrition for growing the pathogen in the laboratory, which would be of immense help for the future scholars. Five different culture media were used to study the growth and sporulation of *Alternaria alternata* causing leaf spot in Giloy. Among them, the Potato Dextrose Agar was found to best support the mycelial growth of the fungus as well as its sporulation. It was followed by Richard's Agar in both the aspects. No earlier work reported nutritional requirement of *Alternaria alternata* causing leaf spot of Giloy. The present work therefore, is bound to serve as a lead for future workers.

SUMMARY AND CONCLUSION

Giloy (*Tinospora cordifolia*) is an important medicinal herb belonging to the family menispermaceae and have multifarious pharmacological uses, because of the bioactive compounds in its plant parts. It is a proven medicine used in *Ayurvedic* and other traditional systems of medicine. Though loaded with lots of advantages, the crop has not gained an expected popularity among the farmers. Recently, Giloy has been listed as an 'important plant' amongst the 32 prioritized plants by National Medicinal Plants Board (NMPB), New Delhi and one among the 33 other medicinal plants to be prioritized by State Medicinal Plants Board (SMPB), Bhubaneswar, Odisha, for the commercialization and popularization of the cultivation techniques among the farmers in different tribal pockets of the state. Giloy can serve as an auxiliary crop to the farmers thereby, generating additional revenue to the growers, an opportunity for doubling farmers' income. With the increasing demand for the crop from pharmaceutical and drug industries, it is high time to increase the acreage of Giloy. For getting an appreciable yield, it is very much necessary to identify the different diseases that are affecting the crop along with suitable and feasible management strategies.

Under this investigation, an attempt has been made to describe the symptoms, identify and characterize the causal agent of leaf spot disease of Giloy, which is a serious disease affecting the Giloy plants in and around Bhubaneswar. For this, diseased leaf samples were collected from five different places near Bhubaneswar namely, AICRP on MAP and Betelvine, Bhubaneswar, Jatni (Khordha), Pipili (Puri), Balipatna (Khordha) and Cuttack. Disease severity was assessed at the respective sites. This was based on numerical rating following 1-9 numerical rating scale, as per the guideline of NICRA for foliar diseases, 2011.

This investigation has revealed that leaf spot of Giloy initiated as small water-soaked lesions turning into round, light brown coloured dot-like spots. These dot-like spots increased in size to become larger circular to oval, dark brown to greyish spots with distinct yellow border initially and dark brown margin later. Spots were more prominent on the ventral side of leaves compared to their dorsal side. Later, the spots enlarged further into round to irregular lesions with concentric rings. The diameter of the spots measured from 2 mm to 5 mm in some blighted leaves. The spots were surrounded by chlorotic zone with greyish white necrotic centers. As the disease

progressed, the greyish center became brittle and papery leading to creation of shot holes. In severe condition, the entire leaf became blighted, causing withering and defoliation of leaves. Disease was found to be prevalent in all the surveyed localities, but disease severity varied between 3 to 8. It was observed that the disease severity was maximum at herbal garden of All India Coordinated Research Project on Medicinal and Aromatic Plants and Betelvine, OUAT, Bhubaneswar, with a disease severity rating of 8, this was followed by Cuttack and Jatni.

Pathogenicity test of isolates from leaf samples collected from different places were done and out of 15 isolates only 9 isolates (TC 1A, TC 2A, TC 2C, TC 3B, TC 3C, TC 4B, TC 4C, TC 5A and TC 5B) could produce the symptoms that were similar to the original sample. The remaining isolates did not produce any symptoms observed under natural conditions and were thus, regarded as saprophytes and excluded from further studies.

An attempt was also made to characterize the virulent isolates, based on their morphological and cultural characters. Except in one case (TC 2C), where black spores covered the entire media surface, in all other cases isolates had white fluffy aerial mycelial growth initially with little variations like creamy and light olivaceous grey colouration when grown on Potato Dextrose Agar medium. At later stage, the mycelial colour changed to greyish black.

The micromorphological studies under a microscope revealed that the hyphae were filamentous, hyaline, multi-celled, septate, irregularly branched, and thin (2.94 µm in diameter) initially but became slightly thick (4.5 µm in diameter) and light grey in colour as they grew old. The conidiophores were straight, erect, septate, golden brown in colour, measuring 42.32 µm in length and 5.12 µm in width. The conidia were borne in chains on conidiophores. They were golden to light brown in colour, beaked and varied in shape from obclavate to mostly muriform, tapering gradually towards apex with 2-3 transverse septa and usually several longitudinal septa.

From the morphological and cultural characteristics of associated organisms, it was unveiled that ***Alternaria alternata*** has been associated with leaf spot disease of Giloy. The identification of the fungal cultures was also confirmed with the help of mycologists of Department of Plant Pathology, OUAT, Bhubaneswar.

An attempt was also made to find out the most suitable source of nutrition for growing the fungal pathogen in the laboratory. Five different culture media viz., PDA, Czapek's dox Agar, Richard's Agar, Oat Meal Agar, Ashby's Agar were used to study the growth and sporulation of *Alternaria alternata* causing leaf spot in Giloy. Among them, the Potato Dextrose Agar best supported the mycelial growth of the fungus which attained a mean colony diameter of 90 mm along with abundant sporulation. This was followed by Richard's Agar, on which the pathogen grew upto 83 mm in diameter and sporulated abundantly. It was noted that Ashby's Agar was least suitable for the pathogen as it showed lowest colony growth and moderate sporulation on the media.

Therefore, it can be concluded that Giloy leaf spot disease noticed in the region (in and around Bhubaneswar) is caused by ***Alternaria alternata***. The disease can be detected/identified on the basis of symptoms described in this literature. To the best of my knowledge, this disease and its causal organism is studied for the first time and the results depicted are novel to the literature. Further, it is suggested to find out management strategies for the disease which would be of practical importance to the farmers.

REFERENCE

Achar S, Parashurama TR and Shivanna MB. 2014. A New record of leaf spot caused by *Xanthomonas campestris* in *Tinospora cordifolia* in India, *International Journal of Current Microbiology and Applied Science*, **3**(1): 269-273.

Achar S, Parashurama TR and Shivanna MB. 2015. A New flat stem disease of *Tinospora cordifolia* caused by Phytoplasma, *Scholars Academic Journal of Biosciences*, **3**(11):957-959.

Ahmad F, Ali M and Alam P. 2010. New phytoconstituents from the stem bark of *Tinosporacordifolia Miers*, *Natural Product Research*, **24**: 926–934.

Ahmad F, Ali M and Alam P. 2014. New phytoconstituents from the stem and leaves of *Tinospora cordifolia Miers, Natural Product Research*, **35**: 1002–1012.

Astutik S, Pretzsch J and Kimengsi JN. 2019. Asian Medicinal Plants' Production and Utilization Potential: A Review, *Sustainability*, 11, 5483.

Chintalwar G, Jain A, Sipahimalani A, Banerji A, Sumariwalla P, Ramakrishnan R and Sainis K (2007). An immunologically active arabinogalactan from *Tinospora cordifolia*, *Phytochemistry*, **52**(6): 1089-1093.

Chowdhury P. 2020. In silico investigation of phytoconstituents from Indian medicinal herb *Tinospora cordifolia* (Giloy) against SARS-CoV-2 (COVID-19) by molecular dynamics approach, *Journal of Biomolecular Structure Dynamics*, pp: 1-18.

Dwivedi R, Sharma V, Agarwal RC and Bhargav S. 2014. Qualitative estimation of Phytoconstituents and in vitro antioxidant potential of *Tinospora cordifolia* stem extract, *International Journal of Biological and Pharmaceutical Research*, **5**(2): 114-119.

Gahukar RT. 2012. Evaluation of plant-derived products against pests and diseases ofmedicinal plants: a review, *Crop Protection*, **42**: 202-209.

Gangan VD, Pradhan P, Sipahimalani AT and Banerji A. 2017. Cordifolisides A, B, C: norditerpene furan glycosides from *Tinospora cordifolia. Phytochem.* **37**(3): 781-786.

Gaur LB, Singh SP, Gaur SC, Bornare SS, Chavan AS, Kumar S and Ram M. 2014. A basic information, cultivation and medicinal use of *Tinospora cordifolia. Popular Kheti,* **2**(3): 188-192.

Gupta S, Singh R and Ashwlayan VD. 2011. Pharmacological activity of *Tinospora cordifolia, Pharmacology online,* **1**: 644-652.

Hanumanthaswamy BC, Rajgopal D, Farooqui AA and Chakravarthy AK. 2010. Insect pests of *Costus speciosus* Linn., a medicinal plant. *My Forum,* **29**(2): 158-160.

Jagetia GV. 2019. Anticancer activity of Giloe, *Tinospora cordifolia* (Willd.) Miers Ex Hook F and Thoms, *International Journal of Complementary and Alternative Medicine,* **12**(2): 79-87.

Jagetia GC and Rao SK. 2006. Evaluation of the antineoplastic activity of guduchi (*Tinospora cordifolia*) in Ehrlich ascites carcinoma bearing mice. *Biol Pharma Bull*, **29**: 460–466.

Jain R and Prasad B. 2014. Karyotype analysis and chromosome number confirmation in *Tinospora cordifolia* (Willd.) Miers. ex Hook. F. and Thomas. A, *International Journal of Pharma and Bio Sciences,* **5**(3): B996-B1001.

Kamalakannan A, Gopalakrishnan C, Renuka R, Kalpana K, Laxmi LD and Valluvaparidasan V. 2007. First report of *Alternaria alternata* causing leaf spot on *Aloe barbadensis* in India. *Australian Plant Disease Notes*. **3**: 110-111.

Khan, M. M., Haque, M. S and Chowdhury, M. S. 2016. Medicinal use of the unique plant *Tinospora cordifolia*: evidence from the traditional medicine and recent research, *Asian Journal of Medical and Biological Research,* **2**(4): 508-512.

Khandagale VG., Ghuge SA and Qureshi AS. 2010. *Tinospora cordifolia*: A medicinal shrub. *Agribios Newsletter*. **IX** (7): 44.

Krupanidhi S, Abraham PK, Venkateshwarulu TC, Ayyagari VS, Bobby NM, John B, Venkata NA and Aishwarya G. 2021. Screening of phytochemical compounds of*Tinospora cordifolia* for their inhibitory activity on SARS-CoV-2: an in-silico study, *Journal of Biomolecular Dynamics*, **39**(15): 5799-5803.

Lohra S and Kumar K. 2019. Comparative analysis on the effect of EMS and MGS on the stomata of *Tinospora cordifolia* (Willd.), *International Journal of Advanced Research*, **7**(10): 807-812.

Maiti CK, Sen S, Paul AK and Acharya K. 2007. *Alternaria alternata* causing leaf spot and leaf blight diseases of some cultivated medicinal plants of lower Gangetic plains of West Bengal, *Journal of Mycopathology*, **45**(1): 132-136.

Mall TP. 2015. Diversity and status of susceptible hosts for Follicolous fungi from north central Tarai forests of U.P. India, *International Journal of Advanced Research in Biological Sciences*, **2**(12): 81-90.

Marimuthu T, Suganthy M and Nakkeeran S. 2018. Common pests and diseases of medicinalplants and strategies to manage them, *New Age Herbals*, pp: 289-312.

Meshram A, Bhagyawant SS, Gautam S and Shrivastava N. 2013. Potential role of *Tinospora cordifolia* in pharmaceuticals. *World Journal of Pharmaceutical Sciences*, **2**: 4615- 4625.

Mishra P, Preya J, Desai S, Patel D and Meshram D. 2014. Phytochemical analysis and assessment of *in vitro* antibacterial activity of *Tinospora cordifolia*, *International Journal of Current Microbiology and Applied Science*, **3**(3): 224-234.

Mittal J, Sharma MM and Batra A. 2014. *Tinospora cordifolia*: a multipurpose medicinal plant-A Review, *Journal of Medicinal Plants Studies*, **2**(2): 32-47.

Pandey DN. 2018. Possible solution of antibiotic resistance in Ayurveda, *International Journal of Ayurvedic Medicine*, **6**(3): 220-224.

Patil D, Gautam M, Mishra S, Kulkarni P, Suresh K, Gairola S, Jadhav S and Patwardhan B.2009. Quantitative determination of Protoberberine alkaloids in *Tinospora cordifolia*by RP-LC-DAD, *Chromatographia,* **7**(8): 78-89.

Pradhan S. 2016. Standardization of Organic Production Technology for Kalmegh (*Andrographis Paniculata* Nees.), *M.Sc. (Fo.) Thesis, Odisha University of Agriculture and Technology, Bhubaneswar, India*. pp 26-29.

Rajkumar N and Shivanna MB. 2009. Ethno-medicinal application of plants in the eastern region of Shimoga district, *Jounal of Ethnopharmacology*, **126**: 64-73.

Rawat N and Roushan R. 2018. Guduchi; a potential drug in Ayurveda, *World Journal of Pharmaceutical Research*, **7**(12): 355-361.

Saha S and Ghosh S. 2012. *Tinospora cordifolia*: One plant, many roles, *Ancient Science of Life*, **31**(4): 151-159.

Sharma U, Bala M, Kumar N, Singh B, Munshi RK, Bhalerao S. 2012. Immunodilatory active compounds from *Tinospora cordifolia, Journal of Ethnopharmacology*,**141**(3): 918-926.

Shree P, Mishra P, Selvaraj C, Singh SK, Garg RCN and Tripathi YB. 2020. Targeting COVID-19 (SARS-CoV-2) main protease through active phytochemicals of ayurvedic medicinal plants – *Withania somnifera* (Ashwagandha), *Tinospora cordifolia* (Giloy) and *Ocimum sanctum* (Tulsi) – a molecular docking study**,** *Journal of Biomolecular Structure and Dynamics.* **142**(5): 250-255.

Shivanna MB, Karthikere G, Achar S, Madhugiri M, Vasanthakumari and Parinitha M. 2013.Phoma Leaf Spot Disease of *Tinospora cordifolia* and its effect on secondary metabolite production, *Journal of Phytopathology*, **162**:302–312.

Sinha K, Mishra NP, Singh J and Khanuja SPS. 2004. *Tinospora cordifolia* (Guduchi), a reservoir plant for therapeutic applications: A Review, *Indian Journal of TraditionalKnowledge*, **3**: 257–270.

Swain SC and Kumar S. 2018. Status and promotion of medicinal and aromatic plants and betelvine in Odisha, A booklet by AICRP on MAP and

Betelvine, *Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India*, pp: 1-37.

Upadhyay AK, Kumar K, Kumar A and Mishra HS. 2010. *Tinospora cordifolia* (Willd.) Hook. f. and Thoms. (Guduchi)- validation of the Ayurvedic pharmacology through experimental and clinical studies. *International Journal of Ayurveda Research*, **1**(2): 112-121.

Varshney V. 2021 Slay those symptoms: How some Giloy can help amid COVID-19, *Downto earth*, pp: 39.

Ved and Goraya. 2007. Demand and Supply of Medicinal Plants in India. New Delhi,*NMPB & FRLHT*, Bangalore, India, pp: 89-117

Vellingiri B, Jayaramayya K, Iyer M, Narayanasamy A, Govindasamy V, Giridharan B, Ganesan S, Venugopal A, Venkatesan D, Ganesan H, Rajagpalan K, Rahman PKSM, Cho S, Kumar NS and Subramaniam MD. 2020. COVID-19: A promising cure for theglobal panic, *Science of the Total Environment*, **725**: 1-18.

Verma, H., Negi, M. S., Mahapatra, B. S., Shukla, A and Paul, J. 2019. Evaluation of an emerging medicinal crop *Kalmegh* [*Andrographis paniculata* (Burm. F.) Wall. Ex. Nees] for commercial cultivation and pharmaceutical & industrial uses: A review, *Journal of Pharmacognosy and Phytochemistry*, **8**(4): 835-848.

Vision 2030. 2011. Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand-387310, Gujarat, India. pp. 280.

Vision 2050. 2013. Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand-387310, Gujarat, India. pp. 318.