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Review: Plant Extract a Novel for Agriculture.

Tansukh Barupal* and Kanika Sharma.

Microbial Research Laboratory, Department of Botany, M.L.S. University, Udaipur-313001 Rajasthan, India.

ABSTRACT

The rising population demand for food poses major challenges to humankind. In favor of facing this challenge humans used enormous amount of chemically synthesize fungicides to control plant diseases because of their diverse use, easiness of synthesis and extreme effectiveness. However, they are not considered as enduring solutions due to their harmful effects on human being as well as soil health so, nowadays focus is shifting in the direction of biological methods to manage plant diseases as they have no adverse consequence on humans as well as environment. The employ of botanicals / natural products for the control of plant diseases is considered as an interesting alternative way to synthetic fungicides due to their no negative impacts on the environment. The present study attempt will be made to develop the plant extract based bio-formulation for systematic control of leaf spot disease of maize caused by *Curvularia lunata.*

Keywords: Plant extracts, Bio-formulation, Antimicrobial activity, Crop plant, Medicinal plants

Corresponding author

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INTRODUCTION

There are two million kinds of living organisms of which fungi constitutes a hundred thousand species [101] from this we can conclude that fungus is omnipresent. It is responsible for food spoilage, crop deterioration and many health hazards.

Plant parasitic fungi cause disease in several economically important crop plants, which lead to enormous losses [131]. Wheat, rice, maize and barley are major cereal grains in world [33]. Maize is an imperative crop and every part of plant has economic value; the grain, stalks, leaves, tassel and cob can be used to prepared large variety of food and non food product [48, 69]. Further maize has good role in animal feed as an important ingredient and it is extensively used in industrial products including biofuels and alcohol production [141].

Maize is susceptible to several fungal and bacterial diseases, out of them leaf spot of maize caused by *Curvularia lunata* is one of the most destructive disease and causes up to 60% losses [3, 65, 89]. According to Li-yan *et al.* [92] the range of yield losses is 10.10% ~ 48.62. and also Significant damages in maize growing region have been reported (International Maize and Wheat Improvement Center, Mexico).

Both chemical and biological methods have been used to prevent damage caused by *Curvularia lunata* in maize. The chemical method is prevalent because of its diverse use and ease of synthesis. Johnston *et al*. [73] controlled maize leaf spot using Trichloromethyl arenethio sulphonates. The efficiency of Bavistin against the fruit rot pathogen was studied by several workers [12,104,130].Tilt and blitox was found to be highly effective at 250 ug/ml and 500 ug/ml concentration inhibiting the growth of pathogen completely. But it has now been realized that chemically synthesized fungicides cause serious environmental problems and are poisonous to non-target organisms also [9,61,62]. Toxicity of fungicide azoxystrobin against fish, invertebrates and fresh water algae has been reported [113,124]. Effect of various chemical fungicides on mammals include cancer (propiconazole and triadimefon), altered reproduction (myclobutanil and triadimefon), and altered hepatic enzymes (propiconazole, triadimefon, and myclobutanil [30,52].

Hence, now a day's focus is shifting towards biological methods to control plant diseases as they have no adverse effect on humans as well as environment. Adeleye and Ikotun [1] reported that the extracts from a wild variety of *Dioscorea bulbifera* L. had some antifungal activity against *C. lunata.* Ulaganathan and Basha[154] investigated that a soil bacterium, *Bacillus* sp. Strain BC121 showed high antagonistic activity against *Curvularia lunata*. Use of herbal fungicides in control plant disease is gradually gaining importance as they are eco-friendly and cost effective [53,116].

Plant based antifungal formulation are now being used to control plant diseases. These formulations contain active compounds/ extracts along with some inert carrier material [115]. Anis *et al.* [7] developed a Na – alginate based bio formulation for management of charcoal rot disease of sunflower. Suprapta and Khalimi [148] evaluated efficacy of plants extract formulation to suppress *Fusarium oxysporium* caused stem rot disease of vanilla seedlings.

Hence in the present study effort will be made to develop the bio formulation using leaf extract of *Lawsonia inermis* for systematic control of leaf spot disease of maize caused by *Curvularia lunata.*

REVIEW

Plant foods are outstanding source of nutrient and energy and play a major role in facing the challenges of growing population. Agriculture is the primary source of food. Wheat Rice, maize, barley, oats etc. are major cereal crops in agriculture. Cereals provide the staple food to almost every country and region. In the world only 5% of starchy staple food comes from root crops (mostly potato, cassava, and yams, depending on climate), whereas the rest is from cereal. Cereal crops are an excellence source of fat soluble vitamin E, which is a vital antioxidant. Whole cereal grains contain 20 to 30% of the every day requirements of the minerals like selenium, calcium, zinc and copper.

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Selected Crop plant: Maize

Maize (*Zea mays* L) is the most resourceful crop with wider adaptability in varied agro-ecologies. Worldwide, it is cultivated on almost 150 m ha in about 160 countries having wider diversity of soil, climate, biodiversity and management practices that contributes 36 % (782 m t) in the total grain production. United States of America (USA), Argentina, China, Brazil, Mexico and India are major maize growing region in world. Among the countries, The United States of America (USA) has the maximum harvest (> 9.6 t ha-1) of maize in the world that contributes nearly 20 % of the total production in the world and is play major role of the US economy Whereas, the average production in Argentina, China, Brazil, Mexico and India are 6.47, 4.85, 3.7, 2.53 and 2.43 t ha-1, respectively.

In India, maize is the 3rd most significant food crops after rice and wheat. It is cultivated throughout the year in different parts of the country for diverse purposes as well as grain, fodder, green cobs, sweet corn, baby corn, pop corn and industrial purposes etc. The major maize growing states that contributes more than 80% of the total maize production are Andhra Pradesh (20.9 %), Karnataka (16.5%), Rajasthan (9.9 %), Maharashtra (9.1%), Bihar (8.9%), Uttar Pradesh (6.1 %), Madhya Pradesh (5.7 %), Himachal Pradesh (4.4 %).

(Source: Directorate of maize research (DMR) Pusa, New Delhi India (An ISO 9001:2008 certified institute)

Origin of maize:

The origin of maize (*Zea mays* L. ssp. *mays*) has been the matter of intense dispute [19, 99]. In this way Mangelsdorf and Reeves [98] give Triparite hypothesis this suggested that maize arose from an extinct wild maize and Beadle proposed that teosinte (*zea* species) was the ancestor of maize (Teosinte hypothesis). The teosinte hypothesis subsequently become broadly accepted among biologist, According to several experts -Beadle [21], Galinat [46], De Wet and Harlan [37], Kato [78] and Doebley [40,41] -teosinte is the ancestor and wild form of maize.

Morphological Characteristics of Maize and Teosinte (Comparative):

The inflorescence present in both Maize and Teosinte, terminating the main culm (main inflorescence) is male and called the tassel. The primary lateral branches in teosinte are normally elongate, and, the inflorescences terminating these branches (primary lateral inflorescences) are
normally male (tassels).On the other hand, in typical maize, the lateral branch is short, and theprimary lateral inflorescence is female [39].

There are following key difference in morphology of the teosinte and maize, respectively, are:

(i) two ranks vs. four (or higher)ranks of cupules,
(ii) Single vs. paired spikelets,
(iii) Hard vs. soft outer glumes,
(iv) Shattering vs.non shattering ears,
(v) Normally male vs. female primary lateral inflorescences, and
(vi) Normally long vs. short primary lateral branches. Other traits , such as the number of ears per plant or the number of cupules per ear, are presumably secondary effects of domestication, as opposed to primary morphogenetic changes involved in the transformation of teosinte into maize.

Place of origin of maize:

It has been considered that Maize is originated on the Pacific slope of mesoamerica, now Mexico and Central America 7000-10,000 year ago [160,162]. Accodding to Mangelsdorf [99] archaeological records suggest that domestication of maize begin at least 6000 years ago, taking place independently in regions of the southwestern United States, Mexico, and Central America.

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Botanical description:

Taxonomic classification:

Maize belongs to the family: Poaceae, in order: Poales.

Systemic position of maize [110]:

Kingdom: Plantae: chlorophyll a and b present and show structural differentiation

Division: Magnoliaphyta: vascular plant with flowers and seeds and; ovules enclosed in an ovary (the angiosperms)

Class : Magnoliopsida (formerly Monocotyledones): one cotyledon present in embryo; Tetramerous flowers;
scattered vascular bundles in stem

Order: Poales: monocots with fibrous leaves, inflower parts reduction and fusion.

Family: Poaceae: hollow-stemmed, fruit a caryopsis; the grasses.

Genus: *Zea:* vigorous grasses with separate carpellate staminate and flower clusters; caryopsis fruit fleshy.

Species: *Zea mays*: corn

Vegetative characters:

Habit: Mesophytic herbs.

Stem: Aerial, greenish, hard and succulent, with nodes and internodes which reaching up to 20-30 cm; the tip of
the stem ending in the tassel.

Root: Fibrous root systems present; on the lower nodes of the stem stilt roots are formed and also crown roots arise from the stem just above the ground level.

Leaves: simple, sessile, alternate, exstipulate, wavy margin and parallel venation. Lower leaves 5-10 cm wide and 50-100 cm long whereas upper leaves narrow short, arranged in two row on stem, with an expanded leaf base with leaf sheath. Kranz anatomy present, it is important character of leaf anatomy, is consistently associated with C_4 photosynthesis (C_4 plant).

Floral characters:

Inflorescence: diclinous inflorescence, with female and male inflorescence arranged on the same plant. The male inflorescence known as tassel is present at the apex (top) of the stem. The female inflorescence known as ears (cob), is present on the tips of lateral branches and developed from the axils [93]. The male inflorescence is made to order a panicle with spikelets in pair; one is sessile in each pairs. Further spikelets are covered by glumes.

The female inflorescence are the ears, it is tightly covered by several layer of leaves, and they so closed-in by them to the stem until the emergence of the the pale yellow silks from leaf whorl at the end of the ears. The emergence of the pale yellow silks is actually elongated stigmas that appear like tufts of hair, at first green and later red or yellow. The pair of spikelets are present on the cob. Which is covered by cupules; they are sessile, having a female flower.

Flowers:

are unisexual, sessile, zygomorphic, epigynous; in every flower there is lemma, palea and two lodicules. Androecium represent three stamens, long anther, dithecous and basifixed. Gynoecium has one locule, one carpel, and single ovule show basal plecentation, silky style and feathery stigma.

Pollination: Anemophilous.

Fruit: Caryopsis referred as grain or kernel.

Seed: Endospermic.

Genome of Maize:

The number of chromosome present in *Zea mays* examined 2n=20[85] and Tito *et al*. [151] reported 2n=20 in genus *Zea*.species *Zea.* The genome of maize is large, being somewhere between 2.3-2.7 Gbp[b] with the no. of gene between 42000 and 56000 genes [10,102,128] and contain one or more supernumerary chromosomes, called B- chromosomes, during the meiosis it do not pair with A chromosomes[74].

The genome of maize is characterized by a high percentage of repetitive DNAsequences, including transposons and retrotransposons [59, 91]. Activator (Ac) and Dissociation (Ds) elements are the best characterized transposons, first described by Barbara McClintock [45].

Types of maize:

Types of maize on the basis of endosperm and kernel composition [36,117,127]:

1. Flint maize: 1. It is predominantly in Latin America and Europe for food.
   2. Hard endosperm present in kernel.
2. Dent maize: 1. It is predominantly in USA for grain and silage. and
   2. Kernel filled with soft endosperm and side and base of kernel's presented hard endosperm
3. Floury maize: 1. It is predominantly in Andean region.
   2. Endosperm mostly composed of soft starch: easy to grind and process into food.
4. Waxy maize: 1. It is preferred for food and industrial uses in East Asia.
   2. Kernel entirely contains amylopectin.
5. Pop maize: 1. It is used in all over world for snack food.
   2. Characteristic feature of kernel is high proportion of hard endosperm rather than other maize kernel.
6. Sweet maize: 1. Grown for green ear (sweet corn).
   2. Has higher sugar content.

Use of maize:

Maize can be used to prepared large variety of food and non food product [48, 69]. Further maize has good role in animal feed as an important ingredient and it is extensively used in industrial products including biofuels and alcohol production [141].

The use of maize is different in different countries. In developed countries, USA, EU, Canada and other maize is used mostly to feed animals directly or sold to feed industries and as raw material for fermentation industries[46,103,106,138]. In developing countries use of maize is variable. In Latin America and Africa the main use of maize is for food whereas In Asia it is used for food and animal feed.

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Importance of maize:

In fact in many countries maize is the basic staple food and an important ingredient in the diets of people. Worldwide, it has been estimated that about 21% of the total grain produced is consumed as food.

In India maize is the third most important food grain after wheat and rice. In India, about 28% of maize produced is used for food purpose, about 11% as livestock feed, 48% as poultry feed, 12% in industry and 1% as seed [2].

Composition of edible portion of maize:

Maize is an excellence crop for food, feed and industrial utilization. The composition of edible portion of maize (dry) is given table [55]:

Composition per 100 g of edible portion of maize (dry):

Moisture	14.9 g
Minerals	1.5 g
Protein	11.1g
Carbohydrates	66.2 g
Fat	3.6 g
Calcium	10 mg
Fibre	2.7 g
Iron	2.3 mg
Calories	342
Potassium	286 mg
Phosphorus	348 mg
Thiamine	0.42 mg
Sodium	15.9 mg
Carotene	90 ug
Sulphur	114 mg
Vitamin C	0.12 mg
Riboflavin	0.10 mg
Magnesium	139 mg
Amino acids	1.78 mg
Copper	0.14 mg

Maize cultivation:

Climate and soil requirements:

Primarily maize is a warm weather crop and it is grown-up in wide range of climatic conditions [68]. Maize can effectively be grown in areas getting an annual rainfall of 60 cm. expanded cloudy period is harmful for the crop but an alternating sunlight and cloud of rain is the most idyllic for its growth and accelerated photosynthetic activity and rapid growth of plants it needs bright sunny days.

In India, it is traditionally grown in monsoon (Kharif) season, which is accompany by high temperature ($<35^0$ C) and rains. Controls moisture and nutrient capacity is most important characters which play important role in cultivation of any crops. The ideal soil types for cultivation of maize is loam or silt loam surface soil and brown silt clay loam having fairly permeable sub soil. Deep fertile soils rich in organic matter and well-drained soils are mainly preferred for grown maize. The range of pH of soil 7.5 to 8.5 is good for crop growth.

Diseases of Maize:

This crop plant is prone to several diseases as following: (CIMMYT, Mexico)

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Diseases caused by fungi: Foliar diseases

Brown spot	*Physoderma maydis*
Crazy top downy mildew	*Sclerophthora macrospora*
Brown stripe downy mildew	*Sclerophthora rayssiae* var. *zeae*
Green ear disease	*Sclerospora graminicola*
Philippine downy mildew	*Peronosclerospora philippinensis*
Java downy mildew	*Peronosclerospora maydis*
Sugarcane downy mildew	*Peronosclerospora sacchari*
Sorghum downy mildew	*Peronosclerospora sorghi*
Maize rusts	the three leaves rusts on maize are common rust, *Polysora* rust, and tropical rust.
Common rust	*Puccinia sorghi*
***Polysora* rust**	*Puccinia polysora*
Tropical rust	*Physopella zeae*
Tar spot complex	*Phyllachora maydis* and *Monographella maydis*
Maydis leaf blight	Teleomorph: *Cochliobolus heterostrophus*
Turcicum leaf blight	Teleomorph: *Setosphaeria turcica*
Carbonum leaf spot	Teleomorph: *Cochliobolus carbonum*
***Anthracnose* leaf blight**	Anamorph: *Colletotrichum graminicola*
Yellow leaf blight	Anamorph: *Phyllosticta maydis* (Teleomorph: *Mycosphaerella zeae-maydis*)
Banded leaf and sheath blight	Anamorph: *Rhizoctonia solani* f. sp. *sasakii* (Teleomorph: *Corticium sasakii,* syn. *Thanatephoruscucumeris*)
***Leptosphaeria* leaf spot**	*Leptosphaeria michotii*
***Phaeosphaeria* leaf spot**	*Phaeosphaeria maydis*
***Hyalothyridium* leaf spot**	Anamorph: *Hyalothyridium maydis* (Teleomorph: *Leptosphaerulina* sp.)
***Curvularia* leaf spot**	*Curvularia lunata*, *C. pallescens,* and *C. maculans*
Gray leaf spot	*Cercospora zeae-maydis, C. sorghi* var. *maydis*
Zonate leaf spot	*Gloeocercospora sorghi*

***Septoria* leaf blotch**	*Septoria maydis*
Eyespot	*Kabatiella zeae* (syn. *Aureobasidium zeae*)
***Macrospora* leaf stripe**	*Stenocarpella macrospora,* syn. *Diplodia macrospora*
Diseases caused by fungi: Stalk rots and smuts:	
***Pythium* stalk rot**	*Pythium aphanidermatum, Pythium* spp.
Fusarium and Gibberella	
Stalk rots	*Fusarium moniliforme* syn. *Fusarium verticillioides*(Teleomorph: *Gibberella fujikuroi*)
False head smut	*Ustilaginoidea virens*
Head smut	*Sphacelotheca reiliana*
Black bundle disease and	*Acremonium strictum*
Late wilt	(syn. *Cephalosporium acremonium*) and *C. maydis*
***Anthracnose* stalk rot**	Anamorph: *Colletotrichum graminicola* (Teleomorph: *Glomerella graminicola*
Charcoal stalk rot	*Macrophomina phaseolina*
***Botryodiplodia* stalk rot**	*Botryodiplodia theobromae*
***Stenocarpella* stalk rot**	*Stenocarpella maydis,* syn*. Diplodia maydis* *S. macrospora,* syn*. D. macrospora*
Diseases caused by fungi: Ear rots	
***Aspergillus* ear rots**	*Aspergillus flavus, Aspergillus* spp.
***Penicillium* ear rots**	*Penicillium* spp.
Fusarium and Gibberella	*Fusarium graminearum (*syn*. F. roseum)*
ear rots	(Teleomorph: *Gibberella zeae*) *Fusarium moniliforme,* syn*. F. verticillioides* (Teleomorph: *Gibberella fujikuroi*
Charcoal ear rot	*Macrophomina phaseolina*
Ergot, horse's tooth	*Claviceps gigantea*
***Nigrospora* ear rot**	Anamorph: *Nigrospora oryzae* (Teleomorph: *Khuskia oryzae*)
Gray ear rot	*Physalospora zeae* (syn. *Botryosphaeria zeae*) (Anamorph: *Macrophoma zeae*)
Common smut	*Ustilago maydis*

***Botryodiplodia* or**	*Botryodiplodia theobromae*
Black kernel rot	
***Cephalosporium* kernel rot**	*Acremonium strictum* (Syn. *Cephalosporium acremonium*)
***Stenocarpella* ear rot**	*Stenocarpella maydis,* syn. *Diplodia maydis, S. macrospora,* syn. *D. macrospora*
***Hormodendrum* ear rot**	*Hormodendrum cladosporoides* (Syn. *Cladosporium cladosporoides*), *C. herbarum*
Diseases caused by bacteria:	
Bacterial stalk rot	*Erwinia chrysanthemi* pv. *zeae,* syn. *Erwinia carotovora*
Bacterial leaf stripe	*Pseudomonas rubrilineans,* syn. *P. avenae, Acidvorax*
Stewart's wilt	*Erwinia stewartii,* syn. *Pantoea stewartii*
Diseases caused by viruses and mollicutes:	
Maize chlorotic mottle virus (MCMV)	
Maize chlorotic dwarf virus (MCDV)	
Maize dwarf mosaic virus (MDMV)	
Maize rough dwarf virus (MRDV)	
Sugarcane mosaic virus (SCMV)	
Maize mosaic virus I (MMV)	
Maize lethal necrosis (MLN)	
Maize stripe virus (M StV)	
Maize streak virus (MSV)	
Maize fine stripe virus	*(Maize rayado fino virus, or MRFV)*
Maize bushy stunt (MBS)	Maize Bushy Stunt phytoplasma, syn. Maize Bushy Stuntmycoplasma
Corn stunt (CS)	*Spiroplasma kunkelii,* syn. Corn Stunt Spiroplasma

Hence this plant is prone to several diseases. Amongst the fungal diseases, leaf spot is common, widespread, destructive and an economical important disease [88].

Leaf spot disease of maize:

Geographical distribution: The leaf spot disease of maize is prevalent mostly in Subtropical and tropical region of the world [25]. It has been reported from various part of the world and first reported from North Carolina and Georgia, later on from Brazil, Nigeria, Thailand,Yugoslavia and Romania. In India

various species of *Curvularia* have been reported to caused disease the species which are *Curvularia indica, Curvularia clavata*,, *Curvularia lunata*, and *Curvularia andropogonis*.

Symptoms of disease:

The disease is characterized by appearance of small (1-6 mm dia.) necrotic or chlorotic spot with light coloured halo [3, 35].

Causal organism:

The causal organism of leaf spot of maize *Curvularia lunata* (Wakker) Boedjn is soil and seed borne pathogenic fungus [3, 25]. It is characterized by production of brown geniculate conidiophores with curved conidia in host tissue and culture media.

Systematic position:

Kingdom: Fungi
Phylum: Ascomycota
Sub-Phylum: Pezizomycotina
Class: Dothideomycetes
Sub-Class: Pleosporomycetidae
Order: Pleosporales
Family: Pleosporaceae
Genus: Cochliobolus
Species: *Cochliobolus lunatus*
Syn. *Curvularia lunata*

Morphological characteristics: *Curvularia lunata* (Wakker) Boedijn

Colony of *Curvularia lunata* is brown, gray, or black, cottony, hairy, or cushion-like and spreads loosely. Conidia 3-5 celled with middle cell enlarged, dark and curved. Hyphae of *Curvularia lunata* are branched and septate. Conidiophores are erected unbranched and septate. The size of conidia measured 18-29×8-10µm in size [38].

Teleomorph: *Cochliobolus lunatus* Nelson & Haasis

When compatible conidial isolates are paired in Sach's agar media teleomorph is produced. [153]. Ascomata (Ascoma (pl= ascomata) An ascus-containing structure (also called ascocarp) are present superficial, globose to subglobose, showingblack in colour, size 250-750 x 250-750 µm, with protruding ostiolar beaks.(190-690 x 60-160 µm) with a hyaline apex.

The ascomata are developed from columnar or flat stromata (Stroma: An often cushion-like mass of fungal cells or closely interwoven hyphae.), tightly adhering to the substrate at the base.

Asci are vestigial bitunicate and appeared almost cylindrical with a short stalk, size often 140-215 x 12.5-19.0 µm. The asci produced among the filamentous pseudoparaphyses which arising from the base of the locule. Ascospores present in asci are flagelliform or filiform, hyaline, narrowing towards either end 125-215 x 2.5-6.3 µm, septate(6-13), parallel or coiled in a certain portion of the ascus.

Quick clue. Stromata are very rarely formed; conidia are 18-32 x 8-16µm, always curved at the third cell.

Mode of transmission and establishment of disease:

As *curvularia lunata* is soil and seed borne pathogen, Kumar and Agarwal [84] determined the seed borne inoculums in discoloured seed using seed component plating and microtomy and detected the *Curvularia lunata* in all components viz. tip cap, pericarp,embryo and endosperm of maize seeds. Zhang [166] has found *Curvularia lunata* on the surface of seed of 5 maize cultivars. Mandokhot and Choudhary [97]

concluded that *Curvularia clavata*, a foliar pathogen of maize is saprophytic on organic debris in the soil, and produces sclerotia near the end of growing season which survive till the favourable condition.

The species of *Curvularia* is present everywhere among the soil and vegetation in temperate region. The spores spread via air and are the common cause of disease in plants [163]. Ellision and Evans [43] have reported *Curvularia* species on itch grass, which is known as major weed in the neotropic, particularly in Graminaceous crops. They described it to be plurivorous and adapted rapidly to new host.

The pathogen of leaf spot of maize invades the host through the stomata and occasionally through wounds and spreads through intercellular hyphae and colonizes the host cells causing necrotic. Series of cytopathological changes occur in host tissue including plasmolysis, degeneration of cell's organelles like chloroplast and vacuole, necrosis of protoplasm and collapse of host cell. The amount of cell wall-degrading enzymes like cellulases, pectinases, and xylanases secreted by *Curvularia lunata* has been found to be related to invagination of pathogen [66]. XiaoQin *et al*. [164] reported that the capacity of spore germination of *Curvularia lunata* is lost if dried for more than 1 h. The required temperature range for infection of maize lies between 15-35^0 C with an optimum temperature of 25^0 C.

Xu *et al.* [165] carried out electrophoretic analysis of protein and cloning of cDNA sequences and reported that Brn1 protein is responsible for melanin biosynthesis and virulence differentiation in *Curvularia lunata*.

Importance of *Curvularia lunata:*

Curvularia lunata is dispersed worldwide particularly in the tropics and is frequently encountered as a pathogen. It causes severe losses in the tropical regions but is a minor pathogen in temperate regions. *Curvularia lunata* is known to produce the metabolites like brefeldin A, anthraquinone, cynadontin, cytochalasin B, D-mannitol and radicinol[] [26,32,112,156,161].

Besides leaf spot disease of maze, *Curvularia lunata* causes severe human diseases including subcutaneous phaeohyphomycosis in a renal transplant patient [158,], endophtalmitis [121], Optic atrophy, fatal systemic infections [29,149].

Bioformulation: Management of Leaf spot disease of maize:

The growing population demand for food poses major challenges to humankind. For facing this challenge humans used vast amount of chemically synthesize fungicides because of their diverse use, ease of synthesis and extreme effectiveness. However, they are not considered as long-term solutions due to their harmful effects on human as well as soil health [90,129].So, nowadays focus is shifting towards biological methods to control plant diseases as they have no adverse effect on humans as well as environment. The use of botanicals / natural products for the control of fungal diseases in plants is considered as an interesting alternative method to synthetic fungicides due to their less negative impacts on the environment [28].

The present investigation focuses on minimizing the dose of chemical fungicides using bio-formulation for systematic control of leaf spot disease of maize.

Available literature shows that wide ranges of chemical substances have been used to control leaf spot disease of maize.

Kumar and Agarwal [86] evaluated thiram , dithane M-45, dithane Z-78, rovral, Bavistin a nd Ridomil-MZ as seed treatment against seed borne pathogens(Cuvularia lunata, Fusarium moniliforme, and Bipolaris maydis) and they found that bavistin and thiram were better for improving seedling vigor of infected maize. Manrao *et al*. [100] studied the fungicidal activity of 4- methoxy benzalaniline and its N-phenyl derivatives against C.lunata and observed that 4 methoxybenzal-(p-toluidine) was most effective. Fungitoxicity of 4-thiazolidinones against spore germination of *Curvularia lunata* was assayed by Sharma *et al*. [136] and they found the antifungal activity was comparable with Mancozeb.

The use of technical grade and wettable powder powder formulation of thiram as seed treatment of maize and seed were tested against spore suspension of *Curvularia lunata, Aspergillus niger, Colletotrichum graminicola, Fusarium moniliforme, Bipolaris sorokiniana, Trichoderma virens and Trichoderma viride*.

Maximum inhibition of *Curvulari lunata* was observed after 48 h of incubation [125]. The toxicity of chlorothalonil, thiram, zineb and mancozeb has been evaluated against *Curvularia lunata* and amongst these thiram was found to be the most toxic against *Curvularia lunata* [17].

The efficacy of fernasan D was tested against seed borne fungi of maize by Ekpo and Benjoko[42] and they reported total control of *Curvularia lunata,Drechslera maydis* and *Curvularia pallescens.* [114] has reported that ridomil-MZ completely inhibited the conidial germination and mycelia growth of *Curvularia lunata*. Grewal and Payak [56] observed *in vitro* inhibition of mycelial growth of *Curvularia pallescens* using cerasan and captan. The persistence of captan was more than zineb on surface of maize leaf [8]. According to Pandurange *et al*.[119] Maneb and Mancozeb were significantly efficient in minimizing leaf blight and grain yields. Issa [70] also reported that spray application of mancozeb was more effective in controlling leaf blight of maize.

The use of botanical / plant product to suppress the leaf spot disease of maize has been found to be effective. However, only few references are available regarding *Curvularia* leaf spot (CLS). Purohit and Bohra [126] examined the antifungal properties of plant extract of *Coriandrum sativam* , *Foeniculum vulgare*, *Anethum graveolens* and *Trigonella foenumgraecum* against *Curvularia lunata*, *Alternaria alternata* and *Fusarium oxysporum* using poison food technique. And found That root extracts of *Foeniculum vulgare* and *Coriandrum sativam* toxic to Curvularia lunata.

The botanical, essential oil and biological agent have been used to *in vitro* manangement of *Curvularia lunata* by Bisht *et al*. [25]. Amongst the plant extract *Lantana* and amongst the essential oil *Citronella* oil were found to be highly effective, whereas, among different strains of *Trichoderma harzianum*, strain Th-13showed maximum mycelial growth inhibition. The *in vitro* and *in vivo* efficacy of plant extract of *Clerodendrum viscosum* was done against seed borne pathogen *Curvularia lunata* of rice. The leaves extract of *Clerodendrum viscosum* significantly increased seedling vigour and reduced infection percentage [123]. Behura *et al*.[22] also reported that leaf and rhizome oil of *Curcuma longa* suppressed the growth of common rice pathogen including *Curvularia lunata*.

Best activity of petroleum ether extract out of petroleum ether, ethyl acetate, chloroform, and methanol extract of *A. indica* seed against *Curvularia lunata* and Aspergillus niger has been reported [159]. Olufolaji [114] studied the *in vitro* and *in vivo* effect of leaf extract of *Azadirachta indica*, *Chromolaena odorata* and *Ocimum gratissimum* against *Curvularia lunata*. Amongst these *Azadirachta indica* showed best inhibition of growth and sporulation. Kishore and Pande [81] evaluated inhibition activity of clove oil, cinnamon oil, and five essential oil compounds like Citral, Eugenol, Linalool Geraniol, and Limonene for 14 phytopathogenic fungi and found complete inhibition of growth of *Curvularia lunata, A. alternata, A. flavus, F. moniliforme* and *Phoma sorghina.* Garhwal [49] evaluated the neem based bioformulation (M/S Godrej Agro vet Ltd., Mumbai) and *Aloe vera* against *Curvularia lunata* by poision food technique.

Review reported on antifungal activity:

Several plant species have been screened for antifungal activity against various plant pathogenic fungi [16,105,118]. Balakumar *et al.* [14] studied the antifungal activity of *Aegle marmelos* (L.) Correa (Rutaceae) leaf extract on dermatophytes. *Aegle marmelos* leaf extracts and fractions were observed to have fungicidal activity against various clinical isolates of dermatophytic fungi. The MIC and MFC was found to be high in water and ethyl alcohol extracts and methanol fractions (200µg/mL) against dermatophytic fungi studied.

Singh *et al.* [145] evaluated the antibacterial activity using plant extract against *E.coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Psuedomonas putida*, *Klebsiella*, *Salmonella*, *Acinetobacter* and *Alcaligen*. Kazmi *et al.* [79] evaluated that Anthraquinones are antibacterial in nature. Javed *et al.* [72] performed the inhibitory effect using agar-well diffusion method against four post-harvest fungi, namely, *Aspergillus flavus* Link ex Gray, *Aspergillus fumigatus*Fres., *Aspergillus nidulans* Eidam ex Win and *Aspergillus terreus*. Kader *et al*.[75] deliberated the effect of aqueous extracts of *Allium sativum*, Nigella sativa and *Lawsonia inermis*. All the three

plants repressed the growth of dermatophytes. Still, the aqueous extract of *Allium sativum* was found to be most effective.

Ganesan [47] reported that the leaf extracts of some plants such as *Cassia mimosoides, Cassia tora* and *Cassia leschenautina* inhibited the spore germination of the phytopathogenic fungi of *Drechslera oryzae*. Srivastava *et al*.[146] have observed that extract of *Catharanthus roseus* reduced the spore germination and growth of *Alternaria alternata* .Bavaji *et al.* [18] studied the effect of plant extracts and fungicides on radial growth of *Alternaria alternate,* the causal agent of leaf blight of sesame. Pal and Kumar [116] investigated the antifungal activity of some weed extracts viz., *Achyranthes aspera, Partheniumhysterophorus, Cannabis sativa, Calotropis gigantean, Chenopodium album,Canada thistle, Phalaris minor, Cynoden dactylon, Argemone maxicana, Ageratumconyzoides*, and *Lantana camera* against *Fusarium oxysporum* causing wilt disease.

Guleria and Kumar [58] reported antifungal activity of *Vitex negundo*, *Zantoxylum alatum*, *Ipomea carnea*, *Thuja orientalis* and *Cinnamomum camphora* against *Alternaria alternata* and *Curvularia lunata* usingbioautography. In bioautography they used lipophilic (dichloromethane) leaf extract. The best result was shown by lipophilic leaf extract of *T. orientalis.* Akinbode [3] evaluated antifungal activity of *Gliricidium sepium, Tithonia diversifolia, Phyllanhus amarus* and *Morinda lucida* against *Curvularia lunata.* Adeleye and Ikotun [1] reported that the extracts from a wild variety of *Dioscorea bulbifera* L. had some antifungal activity against *C. lunata* andalso Sharma and Sharma [135] evaluated antifungal activity of *Lawsonia inermis* against some plant pathogenic fungi including *Curvularia lunata*. Thus Plants are still widely used in controlling diseases around the world.

In the present study attempt will be made to formulate eco-friendly biofungicide from leaf extract of *Lawsonia inermis.*

Selected plant for controlling leaf spot disease of maize:

Plants have been used throughout the world for their preservative and medicinal powers since ancient time It is estimated that there are 250,000 to 500,000 species of plants on Earth. Relatively small percentages (1 to 10%) of these are used as foods by both humans and other animal [27]. Hippocrates (in the late fifth century B.C.) mentioned 300 to 400 medicinal plants [132].The Bible offers descriptions of approximately 30 healing plants [34].

From ancient civilizations, India has been known to be rich in its plants diversity. Plants are potent biochemists and have been components of phytomedicine since time immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. In recent years plants are used to control the growth of several pathogens.

***Lawsonia inermis* Linn.**

Lawsonia inermis belongs to the family Lythraceae is known as mehndi in Hindi, henna in Arabic and grows wild in abandoned areas [107] commonly cultivated in tropical and warm temperate regions as a hedge of garden and Lawns. It is worldwide known as cosmetic agent used to stain hair, skin and nails.

Taxonomic Classification: [111]

Kingdom: Plantae– Plants
Subkingdom: Tracheobionta – Vascular plants
Superdivision: Spermatophyta – Seed plants
Division: Magnoliophyta – Flowering plants
Class: Magnoliopsida – Dicotyledons
Subclass: Rosidae
Order: Myrtales
Family: Lythraceae – Loosestrife family
Genus: *Lawsonia* – lawsonia
Species: *Lawsonia inermis* Linn – henna

Main characteristics: [108]:

Habit: Shrub or small tree,

Stem: 3-5 m tall; much branched shrubs; Bark greenish brown; Branchlets thorny.

Leaves: opposite, broadly elliptic-lanceolate, glabrous, acute at both ends; petiole short.

Flowers: Bright red, 10-12 mm across in axillary penicles; terminal solitary or three flowers in cyme [139]; pedicels short and slender.

Calyx: 3-5 mm long, campanulate; teeth 4, ovate, 2-3 mm long, acute.

Corolla: petal Pale yellow or creamy white, sub orbicular,3 mm long, and as much broad, undulate.

Stamens: 8; inserted in pair on the calyx tube.

Fruits: Capsule; globose, 5-6 mm in diam.; supported by the persistent calyx and tipped with style. reddish-brown.

Seed: Many, trigonous, 2-2.5 mm long, tuberculate, brown.

Native: Middle East.

Flowering and fruiting: April to august.

Medicinal properties:

Different part of *Lawsonia inermis* have been used in traditional medicines as well as cosmetic agent since ancient time. The plant shows great medicinal importance due to its anti-tuberculostatic activity [137], hepatoprotective activity [6], Laprosy and Bronchitis [143], use in leucoderma, herpes and burn bodies [23], intestinal antiplasmodic and uterine sedative effects [142], cytotoxic activity [5], antibacterial activity [24,94,147], anti-inflammatory, antipyretic and analgesic activity [4] and fungitoxic activity [44,152].

Instruments and Techniques:

Solid and liquid media which are used for experimental work will be sterilized in an autoclave (Yorco Scientific Instruments, India) at 121°C, 15 lb for 15 min. For the sterilization of glassware like Petridishes, test tubes etc. hot air oven (Yorco Scientific Instruments, India) will be used. Sterilization will successful achieved by exposure of items to 150º C - 180º C for 2 to 4 hours. pH of the medium will be set by digital pH meter (Systronics 335, India).

The solid material will be dissolved using Hot plate (Remi). Inoculation will be done with nichrome wire loop under aseptic condition of stericlean horizontal laminar flow bench (Model No. DMI 88, Deepak Meditech, India,). Biological oxygen demand (BOD) incubator (Super Deluxe, Yorco Scientific Industries Pvt. Ltd.) will be used for Aerobic incubation at optimum temperature and time required. For the weighing of material Digital balance (Sartorius Model number GE 412) will be used.

Heamocytometer (Rohem Instruments, India Model No. B5 748 Silverlite ISI 0269) will be used for counting of microbial cell no. and cell size will be measured using ocular micrometer (Sigma Inc., Japan) calibrated previously with stage micrometer. Both monocular microscope (Olympus, Germany) and binocular microscope will be used for microscopy while trinocular microscope (Olympus, Germany) will be used for microscopic photography work. Photography work will be done by digital camera (Olympus, Japan, Model No. BX51).
Glass soxhlet assembly will be used to collect partially Partially purified fractions in different organic solvents and The extract will be vaccum dried in JSWG vacuum evaporator.

The separation of secondary metabolites will be achieved using Precoated silica gel 60 F254 TLC plates of uniform thickness (20mm x 20mm) (E-merck) and column chromatography. The thin layer chromatography plates will be observed by UV Fluorescence Analysis Cabinet (Macro Scientific works(R), New Delhi).

O.D. measurements will be taken with the help of digital colorimeter (Range 400 to 700 nm, Naina Solaris Ltd., India) and digital spectrophotometer (Systronics 106, India).

Leaf Area Meter-211 (Range 1cm2 to 200 cm2, Systronic, India) will be used to measure Leaf area and Water bath (Yorco Universal Water Bath, Yorco Scientific Indus. Pvt. Ltd.) will be used for maintained the temperature.

Plant posses various type of secondary like phenols, quinines, flavonoids, essential oils, thymol, alkaloids, sterols, coumarines and triterpenoids etc. these secondary metabolites are responsible for antimicrobial activity [67,76,86]. Researcher all over the world are involved in Screening of plant for search of novel compounds which can be used to control fungal and bacterial diseases of plants and humans.

There are two basic method used to preparing plant extract; cold extraction method [134] and hot extraction method [60], in cold extraction method plant material will be suspended in respective solvent for 24hr. After which decoction is filtered and solvent is evaporated. Dried residue is used as crude extract. Several researchers have used this method for screening the antimicrobial activity of plants [15, 51,96,144]. In hot extraction method successive extraction will be done using Soxhlet apparatus [60,82]. In this process use of different solvents ensure complete extraction of all of plant metabolites with respect to each solvent. Several workers have used this method for screening the antimicrobial activity of plants [120,133,157].

Every plant species has its own set of secondary metabolites and solubility of secondary metabolites requires specific solvents. Phytochemical test are carried out to detect phyto-constituents present in individual solvent fractions. Khadikar *et al.* [80] observed phytochemical properties of water extract of *Boerhaavia diffusa* and *Azadirachta indica*. Jager *et al.* [71] reported the presence of pentacyclic triterpenes in various plants. Tewtrakul *et al.* [150] has reported that ethanolic extract of leaves of *T. peruviana* contains iridoid glycosides, cardiac glycosides, flavonoids and triterpenes, monoterpenes. Patil *et al.* [122] have detected the presence of secondary metabolites in various fractions of *Argemone mexicana Linn*. Several workers have studied phytochemical property of plant extracts by qualitative method [11,13, 155].

In order to separation and identify of individual compound present in the active fraction of plant extract can be done by various methods like TLC, column chromatography, HPLC and gas chromatography etc.[64].

Several workers have investigated antimicrobial activity of active compound isolated from plants. Lee [87] used silica gel column chromatography for evaluate fungicidal activity of volatile compounds isolated from *Acorus gramineus* rhizome, against phytopathogenic fungi. Shiac *et al.* [140] separated the antioxidants from extract of *Taraxacum mongolicum* by using HPLC. Gomez-Alonso *et al.* [54] also performed HPLC with UV- Vis photodiode array (DAD) and fluorescence detection for examination of diverse grape and wine phenolics. Kartal *et al.* [77] isolated Echimidine N-oxide from the root of *Symphytum sylvaticum* and this compound was found to be inhibitory against *Epidermophyton floccosum, Dreschlera rostrata, Microsporum canis, Nigrospora oryzae, Aspergillus niger, Allefsheira boydii,* and *Candida albicans*. Drimane sesquiterpene was found to be inhibitory against *Epidermophyton floccosum* and *Trichophyton rubrum whichnis* isolated a from *Drimys brasiliensis [95]*. Ghahfarokhi *et al.* [50] studied Inhibitory effects of aqueous onion and garlic extracts on growth and keratinase activity of *Trichophyton mentagrophytes*.

MATERIALS AND METHODS

The studies on **''Control of leaf spot disease of maize by leaf extract of *Lawsonia inermis* Linn.''** will be carried out through systematically planned experiments. The details regarding materials and methods to be used during the course of studies are being described here.

COLLECTION OF DISEASE MATERIALS, ISOLATION AND PURIFICATION OF THE PATHOGEN:

Infected leaves will be collected from the maize growing area of Udaipur. Fungus will be isolated from infected leaf by Agar plate method [63]. Leaves will be cut into small pieces showing typical lesion of *Curvularia* leaf spot and surface sterilized with 0.1% mercuric chloride ($HgCl_{2)}$ for two minute, after this the cut pieces of leaves are rinsed thrice in distilled water and transferred on potato dextrose agar (PDA).The plates will be incubated at 28 ± 2^0 C for the 6-7 days. After incubation it will be sub cultured from periphery of mycelia growth zone and will be further purified.

INDENTIFICATION OF CULTURE:

Identification of fungus will be done the basis of direct microscopic examination, cultural characteristics and standard keys available.

COLLECTION OF PLANT MATERIAL AND PREPARATION OF EXTRACTS FOR FURTHER EXPERIMENTAL WORK:

The leaves of *Lawsonia inermis* will be collected from the campus of University College of science M.L.S.U. Udaipur and shade dried at room temperature and then ground in an electrical grinder. The ground material will passed throughout sieve of mesh size 60 to obtain a fine powder which will be used to prepare the Cold extracts as well as hot extracts.

Cold Extraction:

Crude extract will be prepared according to the modified cold extraction method suggested by Shadomy and Ingraff [134]. Cold extraction will be done in water, 50% hydro alcohol as well as absolute alcohol. 20 gm of dried and powdered plant material is suspended in 100 ml of solvent (alcohol/water and 50% hydro alcohol) for 48 hrs. The suspension will filtered through Whatman filter paper no.1, vacuum dried with the help of rotary vacuum evaporator. The dried residue will be used as extract and solvent is recycled.

Hot Extraction:

Serial exhaustive method of solvent extraction will be used for successive separation of different phytochemical constituents present in dried plant material [60, 82]. Solvent series used for successive extraction will be as follows:

Petroleum ether $\rightarrow$ Benzene $\rightarrow$ Chloroform $\rightarrow$ Acetone $\rightarrow$ Alcohol $\rightarrow$Methanol $\rightarrow$Water

In hot extraction method successive extraction will be done using Soxhlet apparatus [60,82]. In this process use of different solvents ensure complete extraction of all of plant metabolites with respect to each solvent. Every time before extracting with next solvent the plant material is dried at temperature up to 50°C in an oven. 40 gm dry plant powder will be kept in Soxhlet extraction unit and extraction will be done separately 280 ml of each solvents.

The extracts (Crude and partially purified fraction) will vacuum dried in a rotary evaporator. The dried extract and fractions will be weighed and their percentage extractive value will be estimated by the following formula:

$$\text{Percent extractive} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Phytochemical Study of *Lawsonia inermis* leaf extracts:

Phytochemical analysis of all extracts will be studied by the qualitative methods suggested by Kokate *et al*.[82]. Tests for Detection of Secondary Metabolites as follows:

Alkaloids:

Presence of alkaloids in the extracts will be tested by performing Mayer's test or Hager's test or Wagner's test. Small amount of extract will be stirred with few drops of HCL and filtered and further filtrate used for alkaloids test, reaction with Mayer's reagent appear a cream colored precipitate; Wagner's reagent gives reddish brown precipitate while Hager's reagent results in formation of yellow precipitate

Volatile Oils:

The presence of volatile oils can be detected by Sudan III test, for this Small amount of extract will be mixed with Sudan III dye and appearance of red color indicates presence of volatile oils.

Saponin:

Foam test will be used to detect presence of saponins. small amount of extract and 20 ml distill water both taken in test tube; then shaken in graduated cylinder for 15 minutes. Development of layer of foam at surface indicates presence of saponin.

Tannins:

Ferric chloride test will be used to determine the presence of tannins. Small amount of extract mixed with ferric chloride and lead acetate in test tube; the development of white precipitate indicated the presence of tannin.

Carbohydrates:

The presence of carbohydrates can be detected by Fehling's test and Molish's test. and. Small amount of extract will be dissolved in 5 ml distilled water and filtered. Small amount of extract will be dissolved in distilled water and filtered and further filtrate used for carbohydrates test. In Fehling's test filtrate will be heated with the same amount of Fehling A and Fehling B solution; the appearance of brick red color indicates presence of carbohydrates. In Molish's test few drops of α-napthol and conc. H_2SO_4 added to filtrate and development of purple color indicates the presence of sugars.

Flavonoids:

Detection of flavonoids will be done using Alkaline reagent test; few amount of extract will be mixed with aqueous NaOH. Appearance of reddish brown color indicates the presence of flavonoids.

Sterols:

Liebermann's test will be used for detection of phytosterols; 2 ml $CHCl_3$ and 1 ml of acetic anhydride 1 ml concentrated H_2SO_4 will be added to small amount of extract development of brown colored ring at junction of two layers shows the presence of sterols.

ASSAY OF ANTIFUNGAL ACTIVITY OF CRUDE EXTRACTS AND PARTIALLY PURIFIED FRACTION OF LAWSONIAINERMIS LEAF EXTRACT:

Assay of antifungal activity of crude and partially purified fractions of *Lawsonia inermis* leaf extract will be done against *Curvularia lunata* by Agar well method and Poison food technique [31,57,109]. Minimum inhibitory concentration as well as minimum fungicidal concentration (MIC, MFC) of the extract of *Lawsonia inermis* will also be studied by two fold serial dilution method.

STUDY OF EFFECT OF *Lawsonia inermis* LEAF EXTRACT ON:

a) Growth and reproduction of test organism.

Inhibitory effect of MIC/MFC will be studied by observing the changes in size, shape and structure of reproductive organs, decrease or increase of sporulation and growth etc.

b) Cyto-morphology i.e. cell /mycelium morphology, size and shape etc. of test organism.

Effect of MIC/MFC of the extract will be studied on various cytological parameters like cell/mycelium morphology, size and shape to understand the mode of action.

ISOLATION OF ACTIVE FRACTION/ MOLECULES OF *Lawsonia inermis* LEAF EXTRACT AND STUDY OF THEIR INHIBITORY ACTIVITY:

Active fractions will be isolated by chromatographic methods such as TLC, Column chromatography, HPLC, GC-MS etc. These fractions will be further assayed for antifungal activity.

IN VITRO* ASSAY OF ANTIFUNGAL ACTIVITY OF VARIOUS ELICITORS AND BINDERS LIKE OIL CAKES, COW DUNG, GUAR GUM ETC., AGAINST *Curvularia lunata.

Assay of antifungal activity of various elicitors and binders like oil cakes, cow dung, guar gum etc., will be done against *Curvularia lunata* by poison food technique.

IN VITRO* ASSAY OF ANTIFUNGAL ACTIVITY OF COMBITION OF EXTRACT AND VARIOUS ELICTORS AND BINDERS LIKE LIKE OIL CAKES, COW DUNG, GUAR GUM ETC., AGAINST *Curvularia lunata.

Assay of antifungal activity of combination of extract and various elicitors and binders like oil cakes, cow dung, guar gum etc. will be done against *Curvularia lunata* by poison food technique

***IN VIVO* (BY POT EXPERIMENTS) STUDY OF PREVENTIVE / PROTECTIVE ACTION OF BIOFORMULATION PREPARED BY USING THE CRUDE, PARTIALLY PURIFIED EXTRACT , LEAF POWDER, BINDER, ELICITOR BASED ON RESULTS OF INVITRO STUDIES IN FOLLOWING COMBITIONS:**

- Crude extract + elicitor + binder.
- Partially purified extract + elicitor + binder.
- Leaf powder + elicitor + binder.

These combinations will be used as foliar spray and seed dressing. Earthen pots of 15cm diameter will be filled with steam sterilized soil. Seeds will be surface sterilized with 0.1% $HgCl_2$ and sown in pots. Both preventive and therapeutic effect of herbal formulation will be assayed. Spore suspension will be used for inoculation. Observation will be taken on different growth parameters like length of seedling, number of leaf per plant and protein and carbohydrate content of test crop. Result will be compared with positive control.

STUDY OF EFFECT OF HERBAL FORMULATION ON CROP PLANT:

The effect of herbal formulation on crop plant in concern with growth parameters will be assayed by standard methods.

STUDY OF EFFECT OF PHYSICAL FACTORS ON ANTIFUNGAL ACTIVITY OF EXTRACT AND HERBAL FORMULATION:

The extract/herbal formulation will be subjected to different temperatures, humidity and light conditions to study the effect of these factors on inhibitory activity.

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