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MONOGRAPH

Allelopathic Effects and Characterization of some Medicinal plant extracts in *Vigna radiata* L. Wilzeck and their efficacy in the control of Charcoal rot disease.

Authored by:-

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Preface

The basis for this research originally stemmed from my passion for developing better methods of data storage and preservation. As the world moves further into the digital age, generating vast amounts of data and born digital content, there will be a greater need to access legacy materials created with outdated technology. It is my passion to not only find out, but to develop the tools to break down barriers of accessibility for further generations.

In truth, I could not have achieved my current level of success without a strong support group. First of all, my parents and my daughter who supported me with love and understanding. And secondly, my guides have provided patient advice and guidance throughout the research process. Thank you all for you unwavering support.

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Glossary

AA	**:**	**Ascorbic Acid**
ABA	**:**	**Abscisic Acid**
APX	**:**	**Ascorbate peroxidase**
Arg	**:**	**Arginine**
BSA	**:**	**Bovine serum albumin**
C	**:**	**Control Plant**
CA	**:**	**Cinnamic Acid**
CAT	**:**	**Catalase**
D	**:**	**Day**
DAS	**:**	**Day after sowing**
DNA	**:**	**Deoxy ribonucleic acid**
EDTA	**:**	**Ethylene diamine tetraacetic Acid**
GB	**:**	**Glycinebetaine**
GPX	**:**	**Guaiacol Peroxidase**
GSH	**:**	**Reduced Glutathione**
GR	**:**	**Glutathione reductase**
His	**:**	**Histidine**
IAA	**:**	**Indole-3-Aceticacid**
Lys	**:**	**Lysine**
M	**:**	**Mole**
NAD	**:**	**Nicotinamide adenine dinucleotide**
NADP	**:**	**Nicotinamide adenine dinucleotide phosphate**
NBT	**:**	**Nitro-blue tetazolium**
PAL	**:**	**Phenylalanine ammonia lyase**
POD	**:**	**Peroxidase**
PR	**:**	**Pathogenesis- related**
PVP	**:**	**Poly vinyl pyrrolidone**
RNA	**:**	**Ribonucleic Acid**
ROO	**:**	**Peroxy radical**
ROS	**:**	**Reactive oxygen species**
rpm	**:**	**Revolution per minute**
SOD	**:**	**Superoxide dismutase**
TBA	**:**	**Thiobarbituric acid**
TCA	**:**	**Tri-carboxylic acid**
UV-B	**:**	**Ultraviolet -B**
w.r.t	**:**	**With respect to**
w/v	**:**	**Weight per Volume**
Wt.	**:**	**Weight**

CONTENTS

1. Introduction

a) Allelopathy

This effect is mainly produced by bio chemicals that are known as allelo chemicals that can have beneficial as well as detrimental effects on the target organisms in a concentration dependent manner. It does not require primary metabolism.This effect is mainly present in plant or plant organs including stems, leaves and fruits. It is a formidable impact on both low and high input cropping system and this technology is more easily transferable to farmers in low input management systems by use of heavy herbicide.

b) Importance of pulses in Agriculture with Abiotic and Biotic Stress

Pulses are grown in different parts of the world. Various types of pulses are grown on large areas of South America, Australia, United States under varied soil and management conditions.Pulses are the main prime source of supplementary protein to daily vegetarian diet; also known as poor man's meat.*Vigna radiata* belonging to order Fables and Family Fabaceae is one of the most important pulse crop. It is rich in vitamins, carbohydrates, minerals, iron, magnesium, potassium, copper, niacin, riboflavin.It is also rich in lysine content making them excellent source of supplementing animal protein.

Mungbean is a warm season crop requiring 90-120 days from planting to maturity. Adequate rainfall is required from flowering to late pod filling to achieve high grain yield. The crop is useful in sustainable agriculture systems for reducing fertilizer need and providing supply nitrogen fertilizer to the agriculture field improving soil structure and providing plant protein. The time of flowering and maturity is shortened under stress compared to well water conditions. The crop yield can be severely reduced under biotic and abiotic stress.

Abiotic stress such as drought, water logging, salinity, high radiation has been presented to be more damaging to plant growth as well as crop production .whereas biotic stress cause plant reduction through various bacterial pathogens, fungi, viruses, insects and other naturally occurring factors .

c) Importance of pulses in Agriculture with *Macrophomina phaseolina* under their control measures.

Macrophomina phaseolina (Tassi goid) is one of the most virulent and destructive pathogen which incite disease in wide range of hosts and is devastating in mungbean, the symptoms produced are seedling rot, collar rot, leaf blight and pod rot . *M .phaseolina* is belongs to the class Coelomycetes, Order Sphaeropsidales and Family Sphaeropsidaceae.

This pathogen infects all plant parts; seed infection ranges from 2-16% causing 11% decrease in grain yield and 15% reduction in protein content and is also a source of primary inoculums for the next season. The pathogen, being soil-borne appears at the seedling stage, is infected through the root and many remain up to maturity stage causing yield considerable loss and problems for disease management (Kumari etral.,2012).It causes decrease

in stem height, root and head weight (Raut.,1983). This pathogen is responsible for seedling blight, root rot and early maturing of crop. The characteristic symptoms appear for the shreeding of plant tissue at the stem and tap of the taproot (Sinclair, 1982; Hoes, 1985).

The resistance to fungicides used several pose serious concerns of acquired resistance like Chloronitrobenzene, Bavistin, Vitavax, Brassicola, Allisan (Pande etal., 1989; Chattopadhyay etal 1990).

d) i) Uses of medicinal Plant *Ocimum sanctum* Linn

Ocimum sanctum Linn is called Queens of herbs. It is one of the holiest and most cherished of many healing and healthy in herbs of the orient. Tulsi is potent germicidal, fungicidal and has antibacterial properties. The leaf are basically used in bronchitis and help in break down the kidney stones and stop the progression of breast and oral cancer and also helps in sinusitis, allergies and migrains.

ii) Uses of medicinal plant *Calotropis procera* (Ait.)Ait.f

Calotropis belongs to Dogbane family. It has various bioactive compounds such as amyrin, amyrin acetate, urosolic acid, cardenolides, calotropagenin. They have cytotoxic effect. The latex of this plant is used in leprosy, eczema, and syphilis and heptatic fever. The roots are basically used in hepatoprotective agent, coughs, syphilis and elephantiasis. Moreover flowers are used in cytostatic, abotifacient, asthma and piles.

iii) Uses of medicinal plant *Astragalous tribuloides* Delile

It is an annual herb. It helps in emollient and demulcent in spleen deficiency symptoms such as diarrhea, fatigue and lack of appetite. It also tonifies the lungs and shortens of breath. It has various bioactive compounds such as polysaccharides, triterpenoid saponins from the root of astragalans, astraglucans and astragalosides I-IV and trigonosides.

2. Review of Literature

a) Medicinal plants and their use to control plant pathogens

India has one of the oldest, richest and cultural uses of herbal medicine. There are 7500 species of medicinal plants used as biotechnological tools are important for the multiplication and genetic enhancement of the medicinal plants.

O. sanctumL. Oil is generally used as antifungal, antioxidant and antistress properties and has antioxidant properties.

C. procera has cytotoxic effects for the treatment of various cancers.

A. tribuloides are generally used in tonifies the lungs and shortens the breath.

b) Pulses and their Importance

Pulses are the rich in proteins. Amino acids and micronutrients. *V. radiata* is also known as Green gram, golden gram due to high content of protein and polysaccharides and has higher Level of nutrients, amino acids, saponins and tannins. The mycorrhizal fungi colonize the plant root. *M.phaseolina* causes root rot and causes red to brown lesions on roots to stem to produce dark mycelia and black microsclerotia. It is a heat tolerant pathogen in temperature range of 60-65 degree range.

c) Charcoal rot Disease

Fungi are unicellular and filamentous living organisms. This fungus belongs to Botryosphaeriaceae family. It is a cushion shaped black sclerotia. It causes seedling blight, root rot, charcoal rot. The pathogen affects the crop with high temperature and water stress occurs in growing season.

Symptoms

Infection of charcoal rot disease cause premature dying, loss of vigour and reduced yield. It is grayish black discoloration and shreeding of plant tissue at stem, top of the taproot with hollowing of stem. The primary infection from microsclerotia in soil under the temperature of 28-35 degree C Initially hyphae grow intercellularly in vascular tissues of plant, mechanical plugging of xylem vessels by microsclerotia occur to break the collar regions and secondary infection occur in roots of healthy plants.

Loss in yield caused by *M.phaseolina*

The fungus is reported in soil, seed and stubble borne. The sclerotis serve as a primary means of survival. This disease is most prevalent during the rainy season and high humidity depend upon the causal agent and host that is controlled by Bavistin.

Factors affecting the infection of *M.phaseolina*

Higher root infections occur in reproductive stage. It occurs in hot and dry weather in growing season with range of 28-30 degree C.

Management strategies

Highly toxicity, chemical fertilizers, soil texture and soil chemicals in human food chain. There are number of strategies that are used to reduce the number of sclerotia in soil. Soil chemistry and structure of microbial communities are responsible for the severity of infection *by M.phaseolina* that can reduce the damage of crops that is caused by charcoal rot. The low soil moisture content is responsible for production of microsclerotia to increase the level of inoculums for subsequent host crop.

Control Measures

Resistance to fungicides is a common problem such as Chloronitrobenzene, Bavistin, Allisan, Topsin M are generally used. Solarization is very effective measure of fungicides. Similarly Tillage reduce the stratification of organic residue on the surface. Captan, thiram, Vitavax or Raxil is used**.**

Biocontrol Measures by medicinal plant extracts

We use neem, cotton, groundnut and sunflower cakes are used to reduce the inoculums level of *M.phaseolina*. There is the bioactive compound against phytopathogenic fungi where methanolic compounds are mostly used. *Calotropis procera* latex is generally used as defense strategy against bacteria and fungi. *Astragalous tribuloides* is generally used in AIDS to increase the the drug potency, poor solubility of drugs and reduces strong toxicity level.

Host plant Resistance

Climatic conditions such as temperature, atmospheric moisture plays a significant role in activation and multiplication of *M.phaseolina*. Host plant interaction determines the two things to their antigenic capability. Hypersensitivity play important role by producing ROS, hydrogen peroxidase and nitrous oxide species. It also involves plant disease resistance pathogens and various harmones involved in cell to cell signaling.

d) Secondary metabolites and plant defense: Polyphenolic compounds

Polyphenol antioxidant ability to help in scavenging free radicals and certain metal chelation reactions such as peroxynitrite and hydrogen peroxide removed from the cell to maintain the healthy metabolic function to diminish the concentration of reactive oxygen species to affect the redox reactions signaling. Several antioxidants are used such as BHA, BHT and TBHQ is widely used.

e) Enzymes in plant defense :

Peroxidase

Peroxidase play important role in plant biochemical defence against microbial pathogen. It contains hydrogen peroxide to restrict the development of phytopathogenic bacteria. It is the compounds to donate the electrons to play the defense against pathogen and used to treat the industrial waste waters to polymerize the anilines and phenols in organic solvent matrices.

Polyphenol oxidase

It play important role in plant defense to invade the pathogens and pests. It is generally used in drug industries and treatment of Parkinson disease.

f) Pathogenesis Related Proteins

PR Proteins increase the resistance of whole plant against a pathogenic attack. They are of seventeen types which are used in many purposes such as plant defense response, to inhibit the growth of fungal pathogens. To inhibits the pathogens such as bacteria and fungi**.**

g) Genetic Basis of disease resistance

There interaction is of three methods by gene for gene model, the matching allele model and the quantitative view of resistance methods. They help to increase the immune response .

h) Allelopathy and plant defense

Allelopathy metabolites is used in controlling the several pathogenic microorganisms such as *O. sanctum*L.,*C.procera* and *Astragalous tribuloides* plant extracts used in *V. radiata.*

3. Materials and Methods

a) Plant Material and their Extraction and secondary metabolite profiling of Plant Extracts

There are three varieties of *V. radiata* such as IPM-02-03, RMG-492, SML-668. The planting was done during kharif season and fresh leaves were collected from the following medicinal plants such as *Astragalous tribuloides, Calotropis procera and Ocimum sanctum*

Extraction and Secondary metabolite profiling of plant extracts.

The bioactive secondary metabolites were extracted in alcoholic, acidic and alkaline solvents. In alcoholic extract 1gm leaves was crushed in 5ml of 80% aqueous methanol, filtered and centrifuged at 5000rpm for 10 minutes. The supernatant was collected.

In aqueous acidic extract 1 gm of leaves was boiled in 0.1 NHCL for 30min. insoluble in water dissolved in 80%methanol.

In alkaline extract 1gm leaves was boiled in 0.1 N HCL for 30 min. and centrifuged at 5000rpm for 10 min. and partitioned by ethyl acetate and dissolved in water.

b) **Antifungal activity of plant extracts against *Macrophomina phaseolina***

Antifungal assay was done by the method of Chakraborty etal 2007 and the antifungal index was calculated by using the following formula (AI%)=1-(Wa-Wb)*100. Where

Wa= wt of fungal mycelia

Wb= wt of experimental set

c) Allelopathic effects of Medicinal plants extracts on seed germination of *V.radiata*

The plant extracts were tested for allelopathic effect on the seed germination of V. radiata. The seeds treated with medicinal plant extracts were germinated in petriplates according to the following experimental set and germination percentage is calculated.

Set1 V. radiata seeds soaked in water for 24 hr and imbibed in medicinal plant extracts for further 24 h, 42h, 72h and placed in petriplates for germination

Set2 V. radiate seeds soaked in medicinal plant extracts for 24hr, 48 hr and 72hr and further imbibed in water for 24 hr and placed in petri plates for germination. After one week the soil was added with suitable concentration of plant extracts.

i) Physiological and biochemical parameters of seedlings of *V.radiata* on *M.phaseolina* infection.

The plantlets of V. *radiata* were observed for disease symptoms. There are morphological traits were determined at 3 weeks for seedling growth and to harvest the biochemical parameters were determined at 3-4 weeks growth stage.

ii) Morphological traits:

Plant height (cm),Leaf stem ratio(cm),Leaf Length(cm),Leaf width(cm), Stem thickness(cm), Pod size (cm), Number of pods.

iv) Biochemical analysis *of V.radiata* L. Wilzeck.

a) **Chlorophyll content:** - ChlA, B and Total Chlorophyll content was determined spectrophotometrically. Homogenate mixture was centrifuged at 10000 rpm for 10 min at 4 degree C and supernatant was collected and take the absorbance by

Chla = 12.7(A663)-2.69(A645)*V/1000xW

Chlb= 22.9(A645)-4.68(A663)xV/1000xW

T. Chl=20.2(A645) +8.02(A663)Xv/1000Xw

b) H_2O_2 Content:-

Leaves were crushed in TCA and sample was centrifuged at 15000 rpm for 5 min. and supernatant was collected and recorded the reading in spectrophotometrically in 390nm.

b) Calculate Total Phenolic content

Leaves were crushed in 80% ethanol. Then centrifuge in 10000 rpm for 20 min and supernatant was collected and cooled. Take different aliquots reading spectrophometrically at 650nm.

c) Enzyme assay:-

Sample is homogeneised and Centrifuged at 8000rpm at 10 min for 4 degree C and supernatant is collected at -20 degree C.

i) Protein content:-

Aliquots of enzyme extract taken in phosphate buffer and take absorbance in 595nm to estimate the protein content

ii) PAL

Homogenised leaves have 5mMmercaptaethnol at centrifuged in 12000 rpm at 20 min. and take abosorbance in 290nm in spectrophotometer.

iii) Superoxide Dismutase assay

It is assayed by phosphate buffer, EDTA, Enzyme extract and NBT and take absorbance at 560nm by using spectrophotometerically.

Iv) Catalase assay

Enzyme extract was added with phosphate buffer and it is calculated by extinction coefficient.

v) Guiacol peroxidase assay

Sample is homogeneised by using phosphate buffer in presence of Hydrogen peroxidase method.

4. Results and Discussion

i) Plant Material

We take three varieties of *V. radiata* that is IPM-02-03, RMG-496, SML668 and take fresh leaves were collected for the extraction of bioactive compounds these leaves are *O. sanctum.,C. procera* and *A.tribuloides.*

Extraction and secondary metaboilite profiling of plant extracts:-

Biochemical tests were performed for screening of bioactive photochemicals such as flavanoids, tannins, Terpenoids tests. In Flavanoid test O. Sanctum L. indicating higher concentration of Flavanoid group. In Tannin test *O. sanctum.* And *A. tribuloides* show maximum tchange of colour is shown as comparison to *C.procera*. Similarly, in Terpenoid test O. sanctumL. Show highest terpenoid content as comparison to other extracts.

ii) Antifungal activity of plant extracts against *Macrophomina phaseolina*

There are three types of medicinal plant extracts are prepared alcoholic, acidic and alkaline of three medicinal plants but in this extracts there is *O.sanctum* L. alcoholic and aqueous acidic extract has maximum bioactive compounds for the control of *M. phaseolina.*

iii) Seed germination of *V. radiata* under in vitro conditions treated with medicinal plant extracts

Germination of *V. radiata* three varieties such as IPM-02-03, RMG-492 and SML-668 in three extracts such as alcoholic, acidic and alkaline extracts. Seeds Soaked in water for 24 hr and imbibed in medicinal plant extracts for 24 h, 48 h and 72 hr and placed in MS medium.

Germination of *V. radiata* three varieties such as IPM-02-03, RMG-492 and SML-668 in three extracts such as alcoholic, acidic and alkaline extracts. Seeds Soaked in medicinal plants for 24 hr and imbibed in water for 24 h, 48 h and 72 hr and placed in MS medium.

In experiment of seed germination of *V. radiata* SML-668 variety of seeds in *O. sanctumL*. alcoholic and acidic extract show highest seed germination rate and has highest antifungal index due to the presences of higher amount of phenolic compounds.

iv) Physiological parameters of seedlings of *Vigna radiata* treated with *Ocimum sanctum* extracts on *Macrophomina phaseolina* infection.

The physiological traits are plant height, leaf stem ratio, leaf length, leaf width, stem thickness, pod size and number of pods was determined at harvest of the control and treated plants. The *O. santumL*. Alcoholic extract and alkaline extract shows maximum height and weight is shown in SML-668 variety as comparison to other variety of extracts. Similarly the number of leaves, leaf length and diameter, number of pods are maximum in number and weight of leaves is maximum in SML-668 variety of mungbean as comparison to other varieties of mungbean of different extracts.

v) Biochemical parameters of seedlings of *V. radiata* on *Macrophomina phaseolina* infection by chlorophyll content, Hydrogen peroxidase method, Total phenolic content, Phenylalanine Ammonia Lyase activity, Superoxide dismutase activity, Catalase activity, Guaiacol peroxidase activity.

a) Chlorophyll content:-

Chl a, Chl b and Total Ch l IPM-02-03, RMG-492 and SML-668 Shows all treated plants show better chlorophyll content than untreated plants showing the efficacy of the extracts. O. sanctum L. Alcoholic 48 hr show high efficacy of Chl a, Chl b and total Chl . Whereas, RMG-492 andSML-668 O. sanctum L. shows 24 hr high efficacy of Ch l a, b and Total Ch l.

b) Hydrogen peroxidase content:-

H_2O_2 content of SML-668 shows the variable response of different *V. radiata* varieties to *M.phaseolina* indicates their differential response and effect of the plant extracts towards resistance to the pathogen.

c) Total phenolic content:-

Total phenolic content of IPM-02-03 , RMG-492 AND SML-668 *O.sanctumL.*alcoholic extract 48 hr shows total phenolic content is maximum as comparison to other extracts of different varieties of mungbean.

d) Phenylalanine Ammonia Lyase content:-

PAL content is maximum. IPM-02-03, RMG-492 and SML-668 O.sanctumL.Alcoholic extract of 48 hr shows maximum Phenyl alanine Ammonia lyase content is maximum in Picokat/mg protein.

e) Superoxide Dismutase activity:-

IPM-02-03, RMG-492 and SML-668 shows *O.sanctumL*. alkaline extract 48 hr shows maximum Superoxide dismutase activity in U/mg as comparison to other extracts such as acidic and alcoholic extracts of mungbean.

f) Catalase content:-

IPM-02-03, RMG-492 and SML-668 shows *O.sanctumL*. alkaline extract 48 hr shows maximum Catalase activity in Nkat/mg as comparison to other extracts of O.sanctum L.

g) Guaiacol peroxidase activity:-

IPM-02-03, RMG-492 and SML-668 shows *O.sanctumL*. alkaline extract 48 hr shows maximum GPX activity in Nkat/mg.There is increase the GPXactivity on treatment nof plants with the alkaline extract.

5. Summary and Conclusion

Mungbean is grown particularly in arid and semi arid regions due to rapid growth and early maturing characteristics and ability not restore the soil fertility too make it valuable crop. Irregular annual rainfall and lack of source management cause severe decrease in crop yield. Several control measures have been suggested for *M. phaseolina* infection and has great potential against phytopathogens. Various plant extracts were tested for allelopathic effect on seed germination of *V.radiata* were infected shows 100% germination is observed.

In present study, all biochemical tests is performed and shows that infected untreated plants were severely affected and cause oxidative stress. All the treated plants perform better than the untreated plants showing the efficacy of the extracts with the moderate increase the treatment of plants with the alkaline extracts.

This work has provided an overview of the physiological and biochemical response of *V. radiasta* under *M.phaseolina* infection and shed light on the putative mechanisms involved in oncrease the tolerance to such biotic stress factors. In the present study, *O. sanctum* L. is observed to prevent *M.phaseolina* infection in V. radiate can be successfully used as biocontrol measure for sustainable agriculture. Targeted functional studies of the important genes and molecular pathways in future may help to unravel their biological function during the course of producing resistance plants.