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Bioefficacy of plant extract on Alternaria leaf spot of soybean (Glycine max (L.) Merr)

KEYWORDS: soybean, Bioefficacy, Alternaria leaf spot

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ABSTRACT *Soybean is the most important oil yielding, leguminous and pulse producing crop. It is grown in both kharip and rabbi season. Infected leaf samples of soybean were collected from the different regions of Marathwada directly on the field. Samples collected in gunny (polythin) bags. Samples were brought in the laboratory. Fungal species isolated by Agar plate (9cm) method. PDA (Potato dextrose agar) used for the isolation. In present studies near about 9 fungal species were isolated which are A.flavus, A. niger, F. oxysporum. F. solani, C. sojina,C. kikuchii, Alternaria alternata, Phoma spp Tricoderma spp etc. But Alternaria leaf spot is the major disease of the soybean causing severe damage to the leaf. Spraying of most of the synthetic fungicides created different types of environmental, biodiversitycal and ecological problems. Treatment gives to petridis contenting Alternaria alternata of different concentration of angiospermic plant extract (10%, 15% & 20%) Allium sativum and Allium cepa brought about significant reduction in disease intensity caused by Alternaria alternata leaf spot on the soybean crop. Angiospermic plant extract have bioefficacy to reduction the incidence of disease in soybean crop. Pharmacologists, doctors, plant pathologist's works on the development of an interesting lead compound into an exploitable product by the plant.*

Introduction

Soybean *(Glycine max (L) Merr)* is one of the edible pulses. It's originated from China and sprayed all over the world. It is highly oil yielding crop. Soybean produce a lot of product as like soya milk, soya cake, soya peat, cattle feed etc. This oil yielding crop is subjected to many diseases caused by nematodes, viruses, bacteria and fungus. Agriculture industry has face a problem arising from the development of resistant in fungal pathogen of soybean crop against use to control. Now a day's scientists observed fungal pathogen causing a large damage on the soybean crop. Hence, crop plant of agriculture values infected by nematodes, viruses, bacteria and fungus, pesticides are playing an important role in controlling diseases and help to increasing yield of soybean crop. Diseases are controlled chemically as well as biologically but biological method is better than the chemical method. Fungus is the mainly pathogen, which damages the foliar parts of the soybean crop. Rust, smut, rot, spot and wilt occur on the leaves and stem of soybean

Use of plant extracts controlling fungal growth known as biological control. Plants have ability to synthesize secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids as well as aromatic oils. Earlier researchers have given more attention towards the exploitation of higher plant products as novel chemotherapeu-tants. Ultimate aim of recent research in this area has been the development to the control strategies to reduce dependency on synthetic fungicides which is harmful, costly and polluted for environment. Use of synthetic fungicides has created different types of problems like environmental, ecological and biodiversity too. Some plants extract act as toxicants to the fungal pathogen. The popularity of botanical pesticides is increasing and some plant products are being used worldwide as green pesticides to control pollution. Plant play as important role in sustainable solutions in agriculture, reduce crop losses, eco-friendly management, easily bio-degradable, organic farming, cheaper source, integrated diseases management etc.

Material and methods

Sample collection

In this research work fungal infected parts of soybean were collected from the different area and locations of Marathwada region of Maharashtra, India was carried out during kharif 2014 and brought in the Department of botany S.M.Dnyandeo Mohekar Mahavidyalaya kallamb, Dist Osmanabad. Surveys of fungal infected parts were done, samples are collected randomly, and fresh infected plant materials were used for the isolation of fungus.

Isolation

Potato dextrose agar (PDA) plate method was comfortable for fungal isolation. Fungal isolation was done by PDA medium. PDA composition was peeled potato 200_{gm}/1lit, dextrose $20_{gm/}$ 1lit and agar 15_{gm}/lit, pH were adjusted by ELICO LI 120 pH meter. Finally PDA sterilized by the autoclave with required glassware's. All glassware's transfer in the laminar air flow MICRO-FLIT (INDIA) along with PDA medium. Infected sample inoculating on growth medium and maintain pure culture of fungal species. Simplified fungi identification key by collage of agriculture and environmental sciences, the University of Georgia and The Illustration of Fungi by D.S.Mukadam *et al. (2005)* used for the identification along with microscopic observation. Major diseases causing *Alternaria alternata* selected from isolates. Identify absolutely species and maintain pure culture for further procedure.

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Preparation and use of plant extract

Present study Angiospermic plants which were medicinally important were selected for extraction. In this plant extraction method selected leaves of the medicinal plants. 100 gm leaves sample collected from the field and washed in the distil water (DW). The basic principles were crushing the fresh plant material with the help of mortar and pestle in 100 ml of DW. These materials were filtered through double folded muslin cloth and make a solvent in beakers. Filtrate further filter through Whatsman NO. 1 filter paper by using funnel. Solution made 100 ml by adding DW. Solution was made 100 % it is ready to further required dilution as like 10%, 15% & 20%. Some researchers centrifuged (20,000 rpm, for 30 min) the filtrate for clarification of the plant extract. Plant tissue homogenization in solvent extraction method widely used. PDA (potato dextrose agar) medium was prepared in conical flask and sterilized medium in autoclave at 15 lb/in^2 pressure for 20 min. Angiospermic plant extracts add in to the PDA medium with continuous starring in 1:1 proportion solvent made for the further procedure. Medium poured in petridis (9 mm dia) pure fungal culture of *Alternaria alternata* grown PDA which is 7-8 days old. Cut the central portion of the petridises containing PDA with angiospermic leaf extract with the help of sterile cork borer in aseptic condition (laminar air flow). Fungal pure cultures were inoculating on the plant extract and PDA containing petridises in aseptic condition. Same condition PDA without extract kept for the control to compare another petridises to check and major diameter or percentage of inhibition (Vincent 1927).

Percentage inhibition calculate by formula

$$PI = \frac{C-T}{C} \text{ X } 100$$

PI = Percentage inhibition, **C** = Control, **T** = Treatment.

Result and discussion

The result presented in table clearly revealed that dip treatment in different concentration of angiospermic plant extracts (10%, 15%, & 20%) *Allium sativum* and *Allium cepa* brought about significant reduction in diseases intensity caused by *Alternaria alternata* leaf spot on the soybean.

Photograph B)

Photograph A)

Photo plates: - A) Isolates of *Alternaria alternata* **from soybean leaf spot.**
B) Microscopic photograph of *Alternaria alternata* **spores.**

Organic compounds in the angiospermic plant extract content as like phenolics, quinones, flavones, flavonoids as well as aromatic oils and nutrients. Which accelerate very fast development and growth of the plants and helping to the plants against fungal attack. Significant improvement was observed in plant growth of soybean due to reduction in pathogenic fungal growth shown below in the tables and graphs.

Table 1) Effect of efficient Angiospermic plant extract against Alternaria alternata.

Treatments	Mean Col. dia.*(mm) at Conc.			Av.
	10 %	15 %	20 %	(mm)
Control	90.00	90.00	90.00	90.00
Parthenium hysterophorus (Parthenium)	52.34	45.21	42.82	46.79
Zingiber officinale (Ginger)	41.18	39.10	35.05	38.44
Lawsonia innermis (Mehandi)	57.10	54.20	50.20	53.83
Curcuma longa (Turmeric)	56.24	51.10	48.80	52.04
Allium sativum (Garlic)	18.30	14.10	12.10	14.83
Allium cepa (Onion)	36.16	33.10	30.60	33.28
Withania somanifera (Ashwagangha)	56.26	51.66	46.60	51.52
Ipomea carnea (Beshram)	56.30	55.18	53.60	55.02
Datura metal (Datura)	58.00	55.10	52.10	55.06
Eucalyptus globules (Eucalyptus)	56.00	53.10	48.80	52.63
Azadirachta indica (Neem)	45.66	43.20	40.33	43.06
Lantana camara (Ghaneri)	56.26	52.80	50.10	53.05
S.E. ±	0.05	0.03	0.06	----
C.D. (P=0.05)	0.15	0.10	0.18	----

% = percentage. dia = Diameter. Av = Average. * = Average of four replication.

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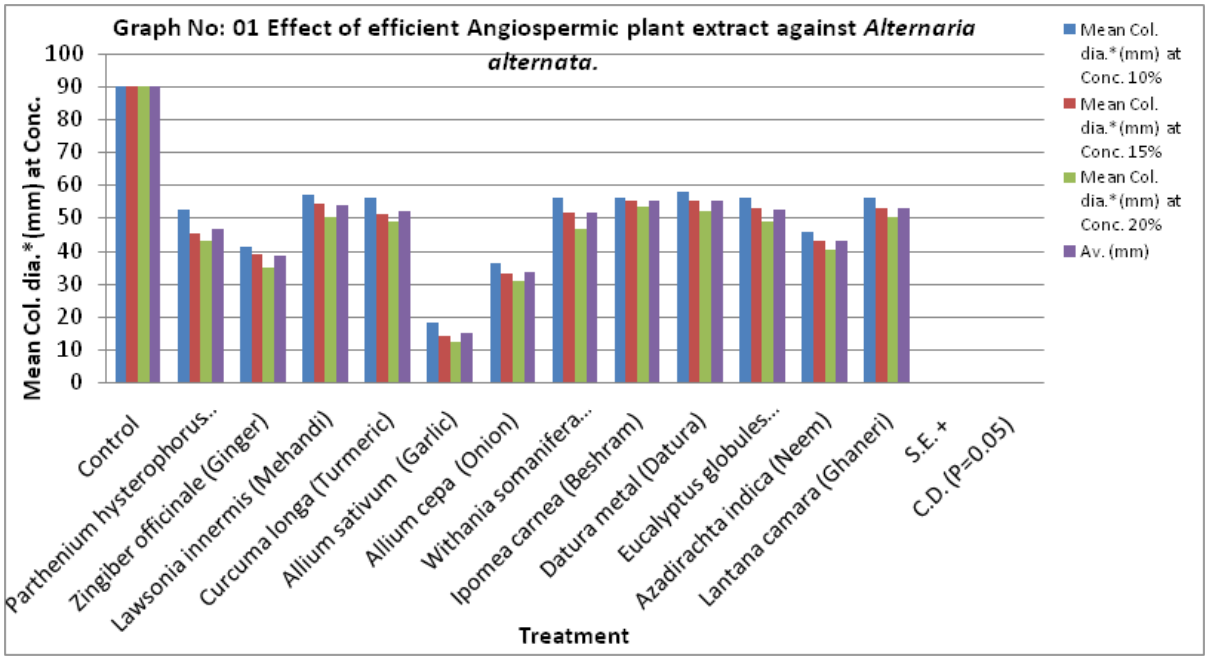

Table 2) Effect of efficient Angiospermic plant extract against *Alternaria alternata*.

Treatments	Percentage Inhibition			Av. (mm)
	10 %	15 %	20 %	
Control	--	--	--	--
Parthenium hysterophorus (Parthenium)	43.97	51.90	52.56	49.47
Zingiber officinale (Ginger)	54.20	58.00	61.01	57.73
Lawsonia innermis (Mehandi)	40.48	40.50	44.20	41.72
Curcuma longa (Turmeric)	39.13	45.15	48.45	44.24
Allium sativum (Garlic)	80.13	84.18	86.85	83.72
Allium cepa (Onion)	61.30	64.10	68.00	64.46
Withania somanifera (Ashwagangha)	38.59	42.46	48.00	43.01
Ipomea carnea (Beshram)	38.00	39.10	41.66	40.10
Datura metal (Datura)	36.50	40.12	43.18	39.58
Eucalyptus globules (Eucalyptus)	36.68	42.20	44.68	41.18
Azadirachta indica (Neem)	46.60	52.15	56.02	51.59
Lantana camara (Ghaneri)	37.48	41.40	45.13	41.33
S.E. ±	0.63	0.63	0.66	----
C.D. (P=0.05)	1.87	1.84	1.93	----

Limitations of biological control in disease management

There are certain limitations in biological control by efficient plant extract as like extraction methods are not standardized. Most studies are in vitro efficiency need the development of formulations. Less effective, less availability and formulations etc are the limitations of the botanical to control and management of the plant diseases.

Conclusion

Plants contain thousands of constituents and are valuable sources of bioactive molecules. The ethno- medicinal knowledge of plant is important for modern day medicine and diet Researcher is investigating for plant products of antimicrobial properties of medicinally valuable plants. Plant extract have bio efficiency in controlling the incidence of disease in crops, plants, animal and human beings. Pharmacologists, doctors, plant pathologists and microbiologists are crucial to see the complete development of an interesting lead compound into an exploitable product by the plant.

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