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Bioefficacy of Medicinal Plant Extract and Pathogenicity of *Verticillium* wilt of Soybean *(Glycine max (L.) Merr.)*

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Abstract: ***Oil yielding crop Soybean also known as miracle crop as well as leguminous and pulse producing crop. It is second most important oil producing crop grown in both kharip and rabbi season also called as cash coop. The presence and significance of phytopathogenic fungal pathogen of soybean wilt was assessed. Gunny bags were used for collection of sample and soybean wilted plant or sample brought in laboratory. Near about 6 different fungal species were isolated from infected sample i.e. Verticillium lecanii. F. oxysporum. F. solani, Alternaria alternata, Phoma spp and Tricoderma spp but Verticillum species occurring as a dominate species among 6 species. Fungi isolated by PDA petridish method. Verticillium lecanii is the entomophilies fungal pathogen also known as Lecticillum lecani. Pathogenicity was calculating by using Koch's postulates. Using of the synthetic chemical fungicides created different types of environmental and ecological problems. Different types of medicinal plant extract used for the treatment to petridish containing fungal pathogen in different concentration (10%, 15% & 20%) plant extract.***

Key words: soybean, bioefficacy, Pathogenicity, medicinal plant extract

1. Introduction

Soybean *(Glycine max (L.) Merr.)* Produce a lot of product as like soya milk, soya cake, soya peat, cattle feed etc is one of the edible pulses. It's originated from China and sprayed over the world wild. Fungi, bacteria, viruses, nematodes etc. is the main cause to loss of yield among these fungi is the main problem of the higher loss. Agriculture industry is the now facing a lot of fungal diseases among these fungal pathogen we are isolated 6 mainly diseases causing fungus *Verticillium* wilt is the newly arising fungal diseases occur in Marathwada region. Before some days *Fusarium* species is the dominant till today but *Verticillium* also occur rarely but it will be problem. 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 crop.

Pesticides are playing an important role in controlling diseases and help to increasing yield of soybean crop. Synthetic chemical fungicides have created different types of problems like environmental and ecological. Biologically control method is better than the chemical method. Some plants extract act as toxicants to the fungal pathogen. Plant extracts use for the controlling fungal growth known as biological control. Medicinal plants have capacity to control fungal growth due to presence of some secondary metabolites as well as aromatic compounds. Earlier researchers have given attention towards the exploitation of higher medicinal plant products as novel chemotherapeu-tants. Chemically control synthetic fungicides which are the harmful, costly and polluted for environment. Popularity of botanical pesticides increasing day by day and some plant products are being used worldwide as green pesticides for control the pollution. Eco-friendly management, easily bio-degradable, organic farming, cheaper source, integrated diseases management etc. is the main purposes of use green pesticide.

2. Material and Methods

Sample collection

In present research work, infected parts of plant were collected from the different area and locations of Marathwada (MS), India. This work was carried out during *kharif* 2014 and bring in the laboratory of Botany Department at S.M.D.M. Collage Kallamb, Dist Osmanabad. Surveys of fungal diseases were done, infected samples of soybean were collected randomly, and fresh infected plant parts were used for the isolation of fungal pathogen.

Isolation

Potato dextrose agar (PDA) method was used for fungal isolation. Fungal isolation was done by PDA medium. PDA composition was peeled potato 200_{gm}/lit, dextrose $20_{gm/}$ lit and agar 15_{gm}/lit, pH were adjusted by ELICO LI 120 pH meter. PDA sterilized by the autoclave 15 lb pressure at 121^0c with glassware's which is required for further procedure. Autoclaved glassware's transfer in the laminar air flow MICRO-FLIT (INDIA) with PDA. Infected sample inoculating on growth medium and pure culture was maintained of fungal species. Simplified fungi identification key by collage of agriculture and environmental sciences, the University of Georgia and *The Illustration of Fungi* by Mukadam D.S *et al.* (2005) used for the identification along with microscopic observation. Among six identified fungal pathogen *Verticillium lecanii* selected from six isolates. Identify absolutely species and maintain pure culture with slants for further procedure.

Pathogenicity of fungi on soybean

Pathogenicity of fungus was test on developmental stage of crop by "Koch's postulate" firstly fungal pathogen isolated by infected sample and maintains pure culture.

PD brought (potato dextrose) 30 ml medium was made and sterilised by the autoclave and pure culture of fungus inoculated on the medium. After 7 days 30 ml PD brought containing fungi kept on shaker for shaking overnight for better mix-up. Next day 1 ml, 2ml and 3ml sample which is good mixture add in to the DW water and volume made 100 ml of each as per concentration 1ml, 2ml and 3ml spores suspension was made. Spore density of sample was calculated by the haemocytometer under the microscopic observation. Calculation was made using formula as below. Healthy plant of soybean sowing in the pot 1.0 % $HGCL_2$ (mercuric chloride) was used for the sterilisation of potted plant and three time washed by distilled water (DW) by sprayer lastly make a wound on plant with the help of blade. Suspension was made in different concentration (1ml, 2ml and 3ml), these suspensions were spread on the sterilised plant and packed by polythin bag and kept in the aseptic condition for further readings.

Preparation and use of plant extract

Medicinal plants were selected for extraction. Plant extraction method fresh and diseases free plant were selected. Sample (100 gm) collected from the field and washed in the DW. Crush the fresh plant material with the help of mortar and pestle in 100 ml of DW finely. Plant materials were filtered through double folded muslin cloth and solvent were made in beakers. Filtrate further filter through Whatsman NO. 1 filter paper by using funnel in funnel stand and 100 ml Solution were made by adding DW. Solutions were made 100 % and solution ready to further required dilution as per different concentration. Centrifuged (20,000 rpm, for 30 min) done the filtrate for clarification of the plant extract by some workers. Solvent extraction method also recommended by some researcher. PDA was prepared in conical flask and sterilized by autoclave at 15 lb/in^2 pressure at 121^{0}c for 20 min. Extracts of different plant material add in to the PDA with continuous starring in 1:1 proportion. Solvent made for the further procedure and medium poured in petridish (7 mm dia) pure fungal culture of *Verticillum lecanii* grown on PDA medium which is 7 days old culture. Central portion of the petridish containing PDA medium cut by the sterile cork borer in strictly aseptic condition (laminar air flow) which were the medicinal plant part extract containing petridish. Pure cultures of fungal pathogen i.e. *Verticillum lecanii* were inoculating on the extract with PDA containing medium in petridish. Same condition PDA medium without extract kept for the control to compare experimental to check and major diameter or percentage of inhibition (Vincent 1927) area of zone.

Percentage inhibition calculate by formula

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

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

Statistical Analysis

Data analysed in this experiments the percentage value transformed in arcsine value. (Standard error) SE and (critical difference) CD and result obtained with compared statistically with the help of SPSS (statistical package for the social science) software was used for the statistical analysis for the obtaining result shown below in the tables.

3. Result and Discussion

The presented result clearly shown that dip treatment in different concentration of medicinal plant extracts (10%, 15%, & 20%) *Spilanthus accemela* and *Madhuka indica* brought about significant reduction in diseases intensity caused by *Verticillium lecanii* wilt on the soybean crop. Organic compounds in the medicinal plant extract content as like flavones, flavonoids, phenolics, quinones, aromatic oils, etc. Because it is accelerate very fast development and growth of the plants and it is also helping to the plant body for preventive weapon against fungal attack. Clearly significant improvements were reported in plant growth of soybean crop due to reduction in fungal growth shown in the tables as below.

Table 1: Effect of Medicinal plant extract against *Verticillium lecanii*

Treatments	Mean Col. dia.*(mm) at Conc.			Av. (mm)
	10 %	**15 %**	**20 %**	
Control	90.00	90.00	90.00	90.00
Semecarpus anacardium (Biba)	42.44	39.31	40.92	40.89
Glycyrrhiza glabra (Jesthmad)	45.28	42.20	39.15	42.21
Spilanthes acmella (Akkalkara)	55.20	52.30	51.35	52.95
Madhuca indica (Mahu)	52.34	52.20	50.90	51.81
Commiphora mukul (Gugul)	35.40	34.30	38.20	35.96
Caesalpinia bonducella	33.26	34.20	32.80	33.42
Vitex negundo (nirgudi)	45.46	42.40	42.56	42.47
Tamarindus indica (Chinch)	42.20	51.28	53.10	48.86
Acacia arabica (Babhal)	42.10	53.20	51.20	48.83
Santalum album (Chandan)	44.20	53.30	50.70	49.04
Azadirachta indica (Neem)	46.46	44.10	42.23	44.26
Aegle marmelos (Bel)	45.46	52.60	53.20	50.42
S.E. +	1.91	2.25	2.15	----
C.D.	0.15	0.04	0.55	----

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

Table 2: Effect of Medicinal plant extract against *Verticillium lecanii.*

Treatments	Percentage Inhibition			Av. (mm)
	10 %	**15 %**	**20 %**	
Control	--	--	--	--
Semecarpus anacardium (Biba)	44.60	40.80	52.80	46.06
Glycyrrhiza glabra (Jesthmad)	55.20	48.10	55.21	52.83
Spilanthes acmella (Akkalkara)	60.23	80.28	82.85	74.45
Madhuca indica (Mahu)	59.20	65.20	69.40	64.06
Commiphora mukul (Gugul)	42.58	50.60	45.30	46.16
Caesalpinia bonducella	40.23	44.05	45.55	43.27
Vitex negundo (nirgudi)	42.49	44.36	50.45	45.76
Tamarindus indica (Chinch)	40.10	40.20	42.60	40.96
Acacia arabica (Babhal)	35.40	41.22	44.25	40.28
Santalum album (Chandan)	36.88	39.20	39.55	38.54
Azadirachta indica (Neem)	45.50	51.25	55.22	50.65
Aegle marmelos (Bel)	36.38	42.60	48.40	42.46
S.E. +	2.74	3.77	3.90	----
C.D.	0.05	0.06	0.06	----

Calculation of different suspension was made by using different formulas likewise.

Spores density/ml =

$$\frac{\text{Average of spores x Dilution factor}}{\text{Volume of a square (ml)}}$$

$$1{:}1 \text{ dilution} = \frac{26 \text{ spores x } 2}{0.0001 \text{ml}}$$

= 520000 spores /ml

Total no spores for 1 ml = 520000 x 1 = 5.2 x 10^5
Total no spores for 2 ml = 520000 x 2 = 10.4 x 10^5
Total no spores for 3 ml = 520000 x 3 = 15.6 x 10^5

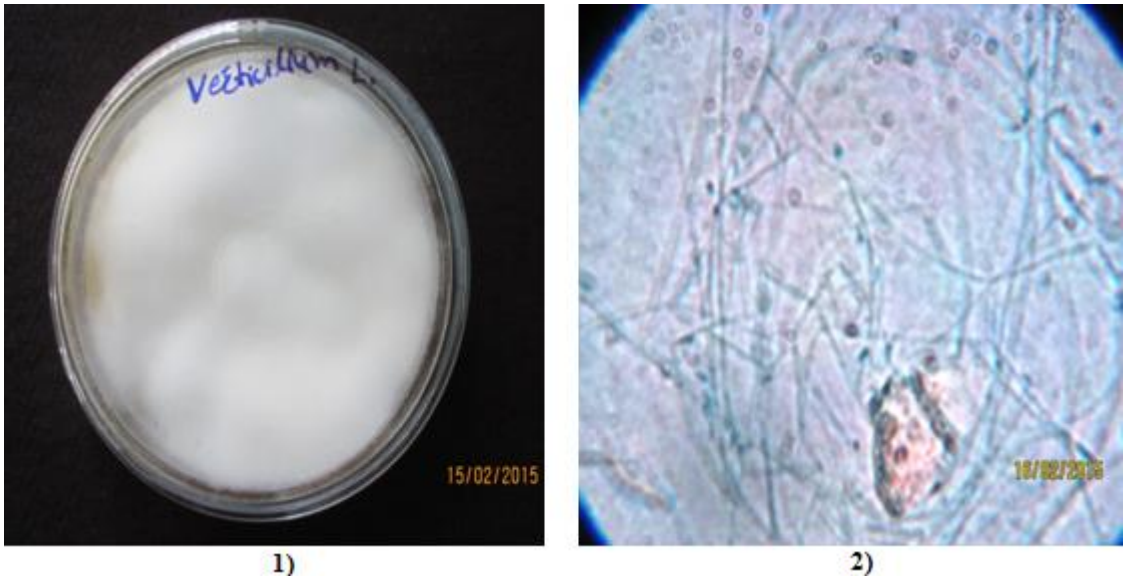
Table 3: Table shows Pathogenicity of *Verticillium lecanii* on soybean crop.

Sr no	Soybean varieties	Different Fungal concentrations		
		1 ml =5.2x10^5 spores	2ml= 10.4x10^5 spores	3ml= 15.6x10^5 spores
1	Control	---	---	---
2	JS 335	+	+	+
3	JS 35	+	+	+++
4	MAUS 71	---	---	+
5	MAUS38	+	+	++
6	PK 1029	---	+	+

Diseases intensity counted as + = 25%, ++=50%, +++=75% and ++++ =100%

A) B)

A) Healthy plant before pathogen applies.
B) Infected plant after pathogen applies.



1) 2)

1) Macroscopic Photograph of fungus *Verticillium lecanii.*
2) Microscopic photograph of *Verticillium lecanii.*

4. Discussion

More than 400 plant species infected by *Verticillium* wilt e.g. soybean, potato, peppermint etc. symptoms occur first on foliar as chlorosis and necrosis beginning in the lower leaves. It is known as "verticillate" (=whorled) arrangement of the phialides on the conidiophorces because it belongs to Deuteromycetes. Conidia are ovoid and usually single-celled and they are borne on phialides which are specialized hyphae. A group of fungi which do not have a known sexual stage called as imperfecti fungi becomes which is saprophytic and colonizes the dying tissues of the host plant. But confirmation of *Verticillium* wilt requires the laboratory techniques it is very simple. Infected pieces of plant vascular cell or tissue kept on to medium which is suitable for growth as like PDA medium incubated for 7-8 days culture grows out of sample it can be identified by the microscopically with the help of manuals. This experiment usually use for accurate identification and diagnosis and scientific study of the plant diseases. Pathogenicity check by the "Koch's postulate" and reading was noted. Plant extract have bioefficacy to control fungal growth as per the result shown in the above table. Biological control will be a great opportunity to the human being to decrees the global worming which is very critical problem this is the essential for protect our planet and life.

5. Conclusion

Medicinal Plants contain thousands of bioactive molecules and constituents are valuable products sources. Ethno-medicinal information of plant is important for modern community as like medicine and diet. Some workers also working and investigating for plant products as well as medicinally valuable properties by the plant. Medicinal Plant extract have bioefficacy in controlling the disease incidence of crops, plants, animal and human beings respectively. Phyto pharmacologists, microbiologists and plant pathologists are crucial to see the complete development of an interesting lead compound into an exploitable product by the medicinal plant. Pathogenicity of fungus also prove by the "Koch's postulate" reading noted in above table. But there is the certain limitations of biological control by medicinal plant extract as like extraction methods are not standardized by the researcher. This methods needs to the appropriate formulations. It is quietly less effective control and management of the plant diseases.

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