ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 07, pp.17758-17761, July, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

PATHOGENICITY OF FUNGI BY "KOCH'S POSTULATES" ASSOCIATED WITH SOYBEAN (*GLYCINE MAX* L.)

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ARTICLE INFO

Article History:
Received 10th April, 2015
Received in revised form
15th May, 2015
Accepted 09th June, 2015
Published online 28th July, 2015

Key words:

Pathogenicity,
Koch's postulates,
Soybean.

ABSTRACT

In the Present research work we were collected infected samples from the field and directly brought in the laboratory of Botany, Shikshan Maharshi Dnyandeo Mohekar Collage Kalamb dist. Osmanabad. Near about 10 fungal species were collected in *kharip,* 2014 from the soybean crop from all over the Marathwada region such as *Fusarium, Alternaria, Penicillum, Rhizoctonia, Tricoderma, Verticillum, Aspergillus, Helmenthosporium, Collatotricum* etc. Isolation of these fungal species was done by PDA (Potato Dextrose Agar) petridish method under aseptic condition (Laminar Air Flow) and maintains pure culture in incubator which was set at 24^0C. Soybean plants cultivated in the plastic bags and growing under controlled and disease free condition in the green house. After 40 days, well growing plant was taken for spraying the fungal suspension. Disease free plant was selected and makes wound on the plants part, with the help of needle and blade. Premade suspension of fungus spread on disease free plant and kept for further results with the control sample. Lastly result were noted + 25%, ++ 50%, +++ 75% and ++++ 100% as like in table 1.

Citation: Shrikant B.Bhosale and D. S. Jadhav, 2015. "Pathogenicity of fungi by "Koch's postulates" associated with soybean (*Glycine max* L.)", *International Journal of Current Research*, 7, (7), 17758-17761.

INTRODUCTION

Soybean is a worldwide important crop which provides oil and protein. Important oil yielding pulse crop is the soybean (Phillip W. Pratt) originated from the China and spread all over the world. In India Madhya Pradesh is the major soybean producing state and Maharashtra is the second one. Range of the diseases affecting of soybean is rather extensive, and caused by fungi are considered as of major importance (Begum *et al.,* 2008). Most of the economically important diseases occurring on the soybean crop are caused by seed borne pathogens (Allen *et al.,* 2010). Soybean has been used in China for 5,000 years for the fixation of nitrogen into the soil as part of crop rotation (ICAR Vision 2030). Defatted soybean meal is a primary, low cost, source of protein for fodder; soya oil is valuable and important product of processing the soybean. The India annual production of soybean is 122.341 lakh metric ton from an area of 120.327 lakh ha with an average yield of 1017 tons per ha during the year 2013 reported by the Soybean Processors Association of India (SOPA). Traditional fermented and non-fermented products of soybean such as soya sauce, soya milk etc. are used as food. Hence the objective of this study was to evaluate the health quality of soybean crop and pathogenicity (Khan and Sinclair1992) of the fungus.

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Pathogenicity were check by the Koch's postulates and recommended diseases causing pathogen isolated from infected sample and grown in pure culture under control condition (Ramesh *et al.,* 2013). Pure culture which was maintained further applying and observing same diseases on plant were studied. Three different type of varieties were selected which is JS 335, JS 9305 and MAUS 71 from Mahabij seed processing unit Dhoki village from Osmanabad dist. Pure culture of fungi was maintained and applying "Koch's postulates" for pathogenicity of the selected fungal pathogen (Hartman *et al.,* 1986). After that artificially infestation was done and result noticed after three to seven days regularly in the tables.

MATERIALS AND METHODS

Sample collection

Infected samples were collected from the different locations of Marathwada (MS), region of *kharif* season (2014). Sample brought in to laboratory of research centre of Botany held in S.M.D.M. Collage Kalamb, Dist. Osmanabad. Samples collected randomly and infected parts were used for the isolation of fungi.

Isolation

PDA (Potato Dextrose Agar) method was used for isolation (Potato 200^{gm}/lit, Dextrose 20^{gm}/ lit and Agar 15^{gm}/lit, pH were adjusted by ELICO LI 120 pH meter). Sterilized medium was used for isolation and infected sample clean by the 70% alcohol in aseptic condition (Laminar Air Flow). Infected sample inoculating on PDA medium and pure culture maintained in the incubator at 24^{0}C temperature. The Illustration of Fungi by Mukadam *et al.* (2005) used for the identification along with microscopic observation as well as some fungus collected from the Agharkar Research Institute, Pune. Among this fungus 10 dominant species were selected for pathgenicity test.

Pathogenicity test

Pathogenicity of fungus was tested 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 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 1ml, 2ml and 3ml sample which is good mixture add in to the DW and volume made. 100 ml of each as per concentration 1 ml, 2 ml and 3 ml spores suspension was made. Healthy seeds of soybean sowing in the black plastic bag and 1.0 % $HGCL_2$ (Mercuric Chloride) was used for the sterilisation of sowing 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 (1 ml, 2 ml and 3 ml), were spread on the sterilised plant and packed by polythin bag and kept in the aseptic condition for further readings.

RESULTS AND DISCUSSION

Healthy growing one month old seedlings of susceptible soybean JS-335 were selected. It was incubated in the polyhouse, where humidity maintained (>80%) and optimum temperature (24 ± 2^{o}C) for further development of disease symptoms occurrence. After 15 days incubation period, typical symptoms of diseases on artificially diseased soybean plants were observed. Microscopic observations made were found similar to that of the organism from naturally diseased soybean plants. Hence, the test pathogen was confirmed of selected diseases, Pathogenicity proved by applying Koch's postulates. Pathogenicity check by the "Koch's postulates" and reading was noted.

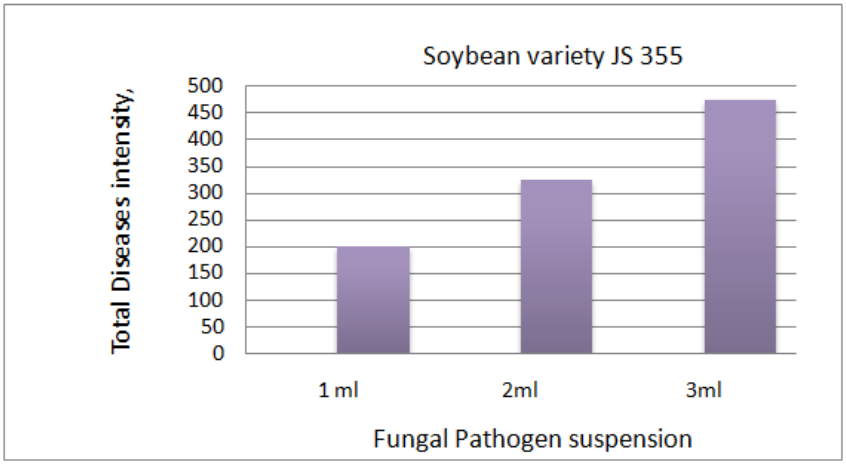
Table 1. Fungal diseases intensity against Soybean variety JS 335

Soybean variety JS 355	Fungal Pathogen suspension	1 ml	2ml	3ml
	Total Diseases intensity	200	325	475

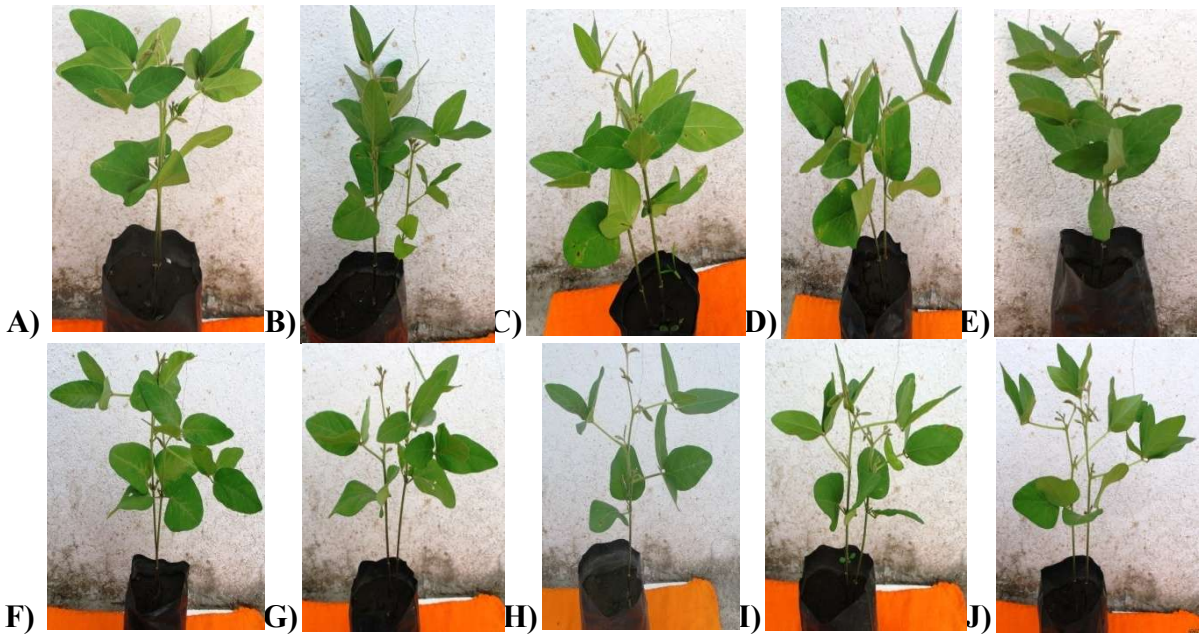
Table 2. Severity of fungal pathogen and its Pathogenicity on soybean crop

S.No	Fungal Pathogen	Soybean variety JS 355		
		1 ml	2 ml	3 ml
1	control	---	---	---
2	*Verticillium* lecani	+	+	+
3	*Rhizoctonia solani*	+	++	+++
4	*Fusarium oxysporium*	--	++	++
5	*Fusarium solani*	+	+	++
6	*Alternaria alternata*	+	++	++
7	*Colletotrichum gloeosporioides*	+	++	++
8	*Aspergillus flavas*	--	--	+
9	*Tricoderma viridi*	--	+	++
10	*Helmenthosporium spp*	++	+	+++
11	*Penicillum notatum*	+	+	+

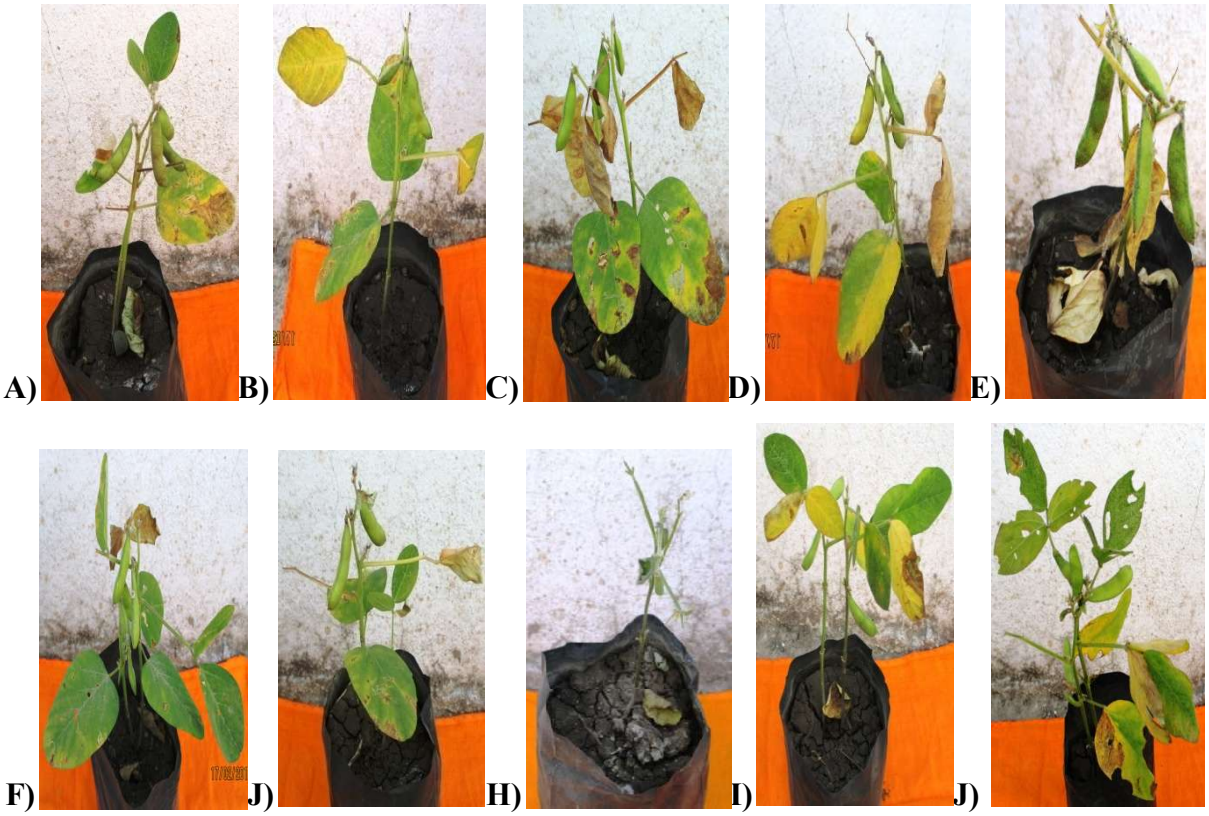
Diseases intensity counted as + = 25%, ++=50%, +++=75% and ++++ =100%



Graph 1. Fungal diseases severity on soybean Varity JS 335



1) Photographs of the healthy plants before infection



2) Photographs of the artificially infected plants

Conclusion

Pathogenicity of fungus also proves by the "Koch's postulate" reading were noted in above table. From the above observation table, it is concluded that Varity JS 335 is very susceptible for *Helmenthosporium spp, Fusarium oxysporium* and *Rhizoctonia solani* but JS 335 is very resistant to the fungi *Verticillium lecani*, *Penicillum notatum* and *Aspergillus flavas*.

Acknowledgments

Authors are graceful to Principal Dr. Ashok D. Mohekar, Shikshan Maharshi Dnyandeo Mohekar Mahavidyalaya kalamb, Dist. Osmanabad, for providing required facilities to this work.

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