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Blight Disease of Linseed with Its Effects on Oil Quality and Ethnomedicinal Control

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Abstract

Linseed crop is common crop of northern Bundelkhand region in Ravi crop, but day by day production rate of linseed and oil quality is decreasing due to some microbial or fungal infection. More than 70% population of this area depend on agriculture and its related job, but due to these infections crop yield is not proper as wanted. Present study have been conducted how *Alternaria lini* effect on oil yield and oil quality. Some biological methods have been given here to cure the crop. Biological methods have been done by using ethnomedicinal plant like *Azadirachta indica, Lantana camara* and *Calotropis procera*, *Chlorodendron*, *Lawsonia*, *Datura*, *Parthenium*, and *Citrus*. *Azadirachta indica* leaves, found more effective than others. There are so many drugs which have been prepared by Linseed to cure high blood pressure and other diseases. Thus linseed is one of the most effective oil yielding plant.

Keywords: *Alternaria Lini, Linum Usitatissimum,* Plant Extracts.

Introduction

Linseed (*Linum usitatissimum L.*) (2n = 30) belong to family Linaceae and genus *Linum*. Oilseed crops occupy an important place in Indian Agriculture. Linseed has numerous medicinal uses. Its fiber is used in the manufacturing of canvas. Cloth, water resistant pipes, paper and strawboard. Linseed oil is used in the manufacturing of paints and varnish, oil cloth and linoleum. India is the fourth largest producer of linseed In India. It is grown in 4.36 lakh hectares with productivity of 1.68 lakh tone after Canada,China and USA. Generally linseed contains 40% oil., 30% total dietary fiber, 20% protein, 4% ash and 6% moisture. The crop is affected by some diseases like *Alternaria* blight, powdery mildew, rust and wilt. *Alteneria* blight caused by *Alternaria lini* is one of the major limiting factors of linseed (*Linum usitatissimum L.*) cultivation in Uttar Pradesh.

The disease appear on all the aerial parts of the plant, resulting leaf and bud blight and ultimately causes substantial losses in yield from 18 to 43.9%. During the routine field and a nearby village's survey, the infection of *Alternaria* blight disease was noticed during flowering stage of linseed plants under field conditions of Bundelkhand. Application of chemical fungicide (carbendazim) against *Alternaria* blight of linseed caused by *Alternaria s* has been reported from kanpur. But the fungicides often lead to serious environmental problem besides affecting the health hazards. So, it is necessary to minimize the use of chemicals for controlling disease. Present experiment was aimed to determine the comparative efficacy of indigenous products on Alternaria leaf blight. There are so many drugs have been prepared by *Linseed* to cure high blood pressure so Linseed is one of the most effective oil yielding plant.

Materials and Methods

Survey and Collection of Samples

A regular and constant survey of linseed crops grown at agriculture fields of Hamirpur, Mhoba and Jhansi districts of Bundelkhand (U.P.) was made. The diseased leaves and bud of linseed showing the characteristic symptoms of different stages were collected in polythene bags. Sterilized throughly with the help of cotton dipped in alcohol then samples were brought to laboratory for examination and isolation of the pathogen. All the work was done in sterilized and aseptic conditions.

Sterilization of Petri Plates and Other Materials

In order to make the experiment free from unwanted microbes sterilization is prerequisite. For the sterilization of glassware, first the petriplates and other glassware were thoroughly washed with detergent, water and then sun dired. After washing petri plates were sterilized in the oven at 160-180^{0}C for 4-6 hours.

Many small instruments like forceps, scalpels, needles, bores etc. were ordinarily sterilized by dipping them in 95% alcohol followed by flaming. These instruments are repeatedly sterilized during the operation to avoid contamination. The mouths of culture vessels were also flamed before pouring or inoculation.

Before inoculation and pouring of the media, hands are repeatedly sterilized with 75% alcohol to avoid contamination.

Laminar air Flow

Inoculation of fungus into the Petri plates was done under laminar air flow. Before each experiment, ultra violet radiations were given on for 15 minutes to kill the microbes. After switching of U.V. radiations, the inoculation was done.

Isolation and Identification of Fungal Pathogen (*Alternaria lini*)

Pathogen was isolated from infected linseed leaves and buds. The infected leaves were surface sterilized with 0.1 percent mercuric chloride (HgCl2) solution, thrice rinsed with sterilized distilled water. Transferred aseptically into petri plates containing melted lukewarm (45^0C) PDA medium and then small pieces of infected leaf were kept aseptically on media inside petri plates. These petri plates were kept in inverted position for incubated at 25 $\pm$ 2^0C in incubator. On 2^{nd} day whitish mycelial colony observed in petri plates and this colony gradually changed into blackish in colour. Some part of colony was taken and slide was prepared by using the method of 1. and observed under the microcrope. Conidiophores *A.lini* were branched, septate, dark in colour and produced mariform conidia.

Pathogtenicity Test (Maiti et al., 2007)

The pathgenicity test of the isolated fungus was made on healthy bud/leaves of host plant in order to establish the pathogenic nature of the fungus. The pathogenic nature of the fungus was tested according to Koch's postulates. For pathogenicity test : plants were grown in sterilized pots by sowing sterilized healthy seeds of linseed variety Chambal.

Biological Control

Effect of Some Plant Leaf Extracts on Mycelial Growth

Relative efficacy of 08 plant extracts *Azadirachta indica, Clerodendron fistulosumm, Datura, Lawsonia alba, Citrus lemon*, belonging to different familiess were tested under laboratory conditions. The efficacy was judged by extent of their inhibitory effects on growth of pathogen on P.D.A medium.

For this purpose the fresh leaves were separately washed in distilled water and were pulverized with sterile distilled water at 1 : 1 w/v in a pestle and mortar and filtered through muslin cloth. This formed 100 per cent plant extract solution which was sterilized in an autoclave at 121.5^0 C and 15 lbs for 20 minutes, to avoid contamination. Requisite quantity of plant extract was added in the medium by using sterile pipette to get 10 percent concentration of plant extract in medium prior to pouring in Petri dishes. Circular discs of 5 mm circles were'cut from 7 days old culture by cork borer. One such disc carrying fungus was placed at centre of each Petri dish containing solidified medium Petri plate without plant extract served as control. Three replications were kept for each treatment at 25 $\pm$ 2^0 C. After incubation for 7 days, the diameter of fungal colony was measured in mm in each treatment regularly every day till the colony growth was full in control plates. The efficacy of plant extract was determined against growth of pathogen in control plates. The percent inhibition over control was calculated by the following formula (Vincent, 1974).

$$\text{Percent (\%) inhibition over control} = \frac{C - T}{C} \times 100$$

C = Growth of fungus in control.
T = Growth of fungus in treatments

Results

The leaf extract of neem are most affective to control *Alternaria lini* causes the disease of linseed. It inhibited 73.56 % growth of fungus over control. It was followed by *Lantana*, *Ocimum sanctum, Calotropis procera, Tridax procumbens, Jatropha gossypifolia, Datura, Parthenium, Euphorbia hirta* and *Ricinus communis* which significantly inhibited the growth of the pathogen. The never ending and imbalance use of synthetic fungicides for controlling the diseases not only harms the consumers but also polluted the environment directly or/and indirectly causing number of non-curable diseases in human being and decrease the efficiency of doing work. Hence it is apparent to find out alternate and safe source of disease controlling agents. Use of botanicals extracts and development of resistant cultivars are the safer mode of controlling the hazardous effects of chemicals or synthetic fungicides.

Alternaria lini cause a brown and black target like lesion on the leaf and buds. Lesions can cover the whole leaf surface and lead to curled and dried of bud of linseed which cause failure of flower to open during the day (Fig. No.1 & 2)

Fig. 1 Symptoms on Leaves of Linseed Plants

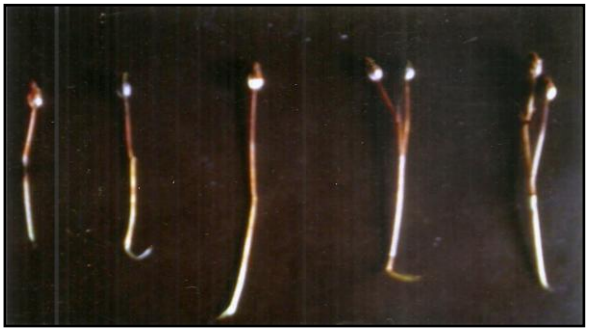

Fig. 2 Symptoms on Buds of Linseed Plants

Identification

Identification of the isolated pathogen was done on the basis of morphological characters and the characters were same as reported by Dey, (1933) For *Alternaria lini* the morphological characters of the isolated pathogen are given below (Fig No. 3):

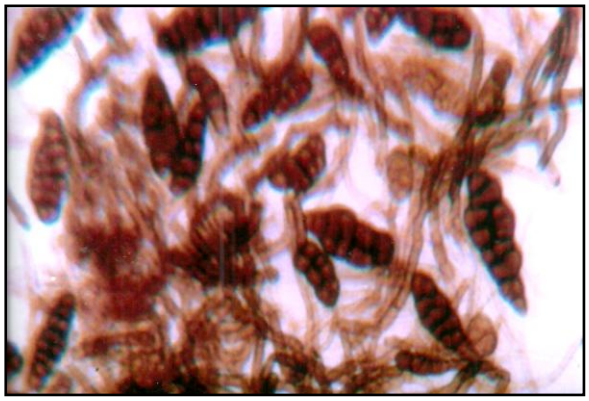

Fig.3 Conidiophore Bearing Conidium of *Alternaria lini*

Hyphae

Septate, branched, hyaline later turning to pale then olive grey. The mycelial width was 3.0 – 5.3 μm.

Conidiophores

Septate, erect, branching or non-branching geniculte, olive buff to dark olive buff, 21.5 – 100.5 μm wide.

Conidia

Conidia are formed simply or in branches chain, consisting 02-10 spores, smooth or verculose, linear to obclavate, dark olive to buff in colour, provided with 1-7 cross and 1-5 longitudinal septa and often with short conical or cylindrical beak, light brown to dark olive buff, measuring 1.5-45.6 x 7.0 – 3.5μm in size, beaks usually light colour measuring 3.5-18.0 x 3.0 – 65. μ m.

Pathogenicity

The pathogenicity test of the purified isolate (Fig. No.4) was carried out on cotyledon stage at 15 days and 75 days old plants (bearing buds and flowers) of linseed variety Chambal. The plants were raised from the surface sterilized seed in pots, filled with autoclaved soil by the technique described under materials and methods. Ten plants per pots were raised and three replications were taken for the study. Some leaves were inoculated by the pathogen by spraying.

The inoculated plant were covered with polythene bags for 24 hours and then placed in laboratory. The leaves and buds were watched daily for the development of disease symptoms. In present study the percentage disease severity was recorded 40-60% in case of leaves after 48 hours of inoculations (Table No. 1)

In case of buds the percentage disease severity was recorded 40%. The symptoms resumed their natural appearance within 48 hours after inoculation (Table No. 2 & Fig No. 4)

Table - 1
Percentage Disease Severity on Linseed Leaves Inoculated with "Alternaria lini"

Treatment	No of leaves inoculated	No. of leaves infected	Disease severity (%)
Upper surface	70	34	48.57 %
Lower surface	70	38	54.28 %
control	70	Nil	Nil

Table - 2
Percentage Disease Severity on Linseed Buds with "Alternaria lini"

Treatment	No of leaves inoculated	No. of leaves infected	Disease severity (%)
Inoculated buds	60	28	46.66 %
Control	60	Nil	Nil

Fig. No. 4 Inoculated Plants with Alternaria lini

Biological Control

The radial growth of the fungus was measured in mm along with control and result are presented in (Table No. 3 & Fig. No. 5) and it is apparent from the result presented in the table that the plant extracts were significantly superior over control in checking the growth of the pathogen. The neem leaf extract was most effective in inhibiting the growth of the pathogen Average colony growth was 90mm in control which was reduced to 25 mm in neem extract. The other leaf extracts which inhibited the radial growth of the pathogen in descending order were *Lantana, Lawsonia, Clerodendron and Citrus.*

All above leaf extracts showed inhibitory potential in checking the growth of *Alternaria lini* and differed significantly from one another as compared to control. The percent inhibition was highest in Petri plates containing neem extracts in comparison to that of other botanicals that is it was 14.44% in citrus and 72.22% in neem other were in between the two ranges.

Table - 3
In Vitro* Effect of Plant Extract on The Growth of *Alternaria lini

Sr. No.	Botanical Name of Plant	Family	dose in (%)	Plant part used	Average colony growth of fungus (mm)	Percent inhibition
1.	*Azadirachta indica*	Meliaceae	10	Leaves	25	72.22
2.	*Clerodendron fistulosumm*	Verbenaceae	10	Leaves	30	66.66
3.	*Datura festoosa*	Solanaceae	10	Leaves	38	57.77
4.	*Lantana camara*	Verbenaceae	10	Leaves	40	55.55
5.	*Parthenium hystrophorus*	Compositae	10	Leaves	42	53.33
6.	*Calotropis gigantia*	Asclepidaceae	10	Leaves	50	44.44
7.	*Lawsonia alba*	Lathyraceae	10	Leaves	75	16.66
8.	*Citrus lemon*	Rutaceae	10	Leaves	77	14.44
9.	*Control*				90	

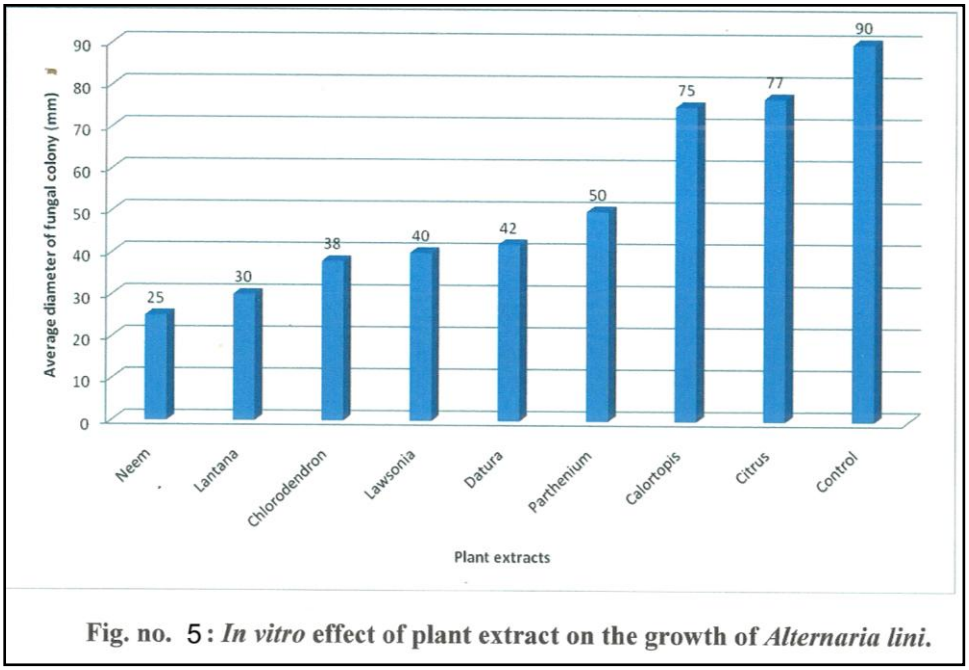

Fig. no. 5 : *In vitro* effect of plant extract on the growth of *Alternaria lini*.

Discussion

The disease symptoms initially appear as light brown to black dots like spots on stem and leaves. Minute dark brown to black spots appeared near the base of calyx, which enlarged later, differed in colour and spread all over the bud passing in to the pedicle the symptoms were same as reported by Dey. 1933.

The pathogen was isolated and indentified as *Alternaria lini* because, its morphological characters which were closely resembling with the description given by Dey. (1933).

During the pathogenicity test of the leaf spot and black bud disease, pathogen (*Alternaria lini*) has been found to parasitize the leaves, floral organs and buds causing seedling blight leaf spot and black bud. The most common symptoms of this disease were leaf spot and black bud. The affected buds in most of the cases were completely replaced by fungal mycelium and conidia. The affected capsule may contain deformed, discoloured and blighted seeds. Similar type of symptoms were observed by Dey. (1933)

The pathogenic fungus (*Alternaria lini*) was isolated from the affected tissues on potato dextrose agar medium. The isolated pathogen produced a similar cultural character which was similar to the description given by Siddiqui, (1963) and Simmons, (1967).

The efficacy of leaf extract of eight plants was tested for control of *Alternaria* blight pathogen in laboratory as well as in field.

The most effective result was with Neem followed by *Lantana, Clerodendron Lawsonia, Datura, Parthenium, Calotropis* and *Citrus*. All plant extract were able to control disease in field also.

Aqueous leaf extract (2%) of eight locally available plants were *in vitro* evaluated against *Alternaria lini,* the causal organism of leaf and bud blight in linseed. Maximum inhibition was recorded with *Azadirachta indica* according to Singh and Singh, (2007).

Lantana camara and *Calotropis procera* were reported to have fungicidal properties against *Alternaria alternata and Fusarium oxysporum* (Bansal and Gupta, 2000). Gungi toxicity of Datura metel was earlier reported by Singh and Tripathi, (2000).

References

1. Anonymous (2010). Annual report: 2009-2010 Linseed, P.C. Unit (Linseed), C.S. Azad Uni. of agri. and tech. Kanpur. pp : 272.
2. Kamalkannan, A; Shanmugam, V.; Surendran, M. and Srinivasan, R. (2001): Evaluation of plant

extract against *Pyricularia oryzae. Ann. Pl. Protect, Sci.,* Vol. 9 (1) P. 68-72.

3. Karalova, J; Jajslovaj, Poustka J.; Hochman, Bjelkovam and odstricilova, L. (2006) : Occurrence of *Alternaria* toxins in fiber flax, linseed, and peas grown in organic and conventional farms : monitoring pilot study Czech *J. Food Sci.* Vol., 24 : pp. 288-296.
4. Karkhi, S. A.; Srivastava, R.L.; Singh, Jyoti ; Malik, Y.P,; Husain K.; Dubey, S. D. and Rai, J., (1999) : Linseed technology for increasing production (Ed.) Hegde, D.M.; Karkhi, S.A. and Pati, D. Director of *oilseed research* Rajendra Nager, Hyderabad, pp. 125.
5. Kerkhi, (2004) : Linseed technology for increasing production oilseed research Rajendra Nagar Hyderabad pp. 125.
6. Khan, M. N.; Singh, R.; Mishra, L.K., Awasthi, L.P., (2009): Efficiency of fungicides against *Alternaria* blight of linseed *journal CABI,* Mhtmi : L file : Documents % 20 and settings/chandrka 5/local%20 setting temp/sharm.
7. Larsen, T.O.; Sevendsen, A. and smedsgaards, J., (2001) : Bio – chemical characterization of ochratoxin a. producing strains of the genus penicilium. Appl. Environ. Microbial., Vol. 67.P. 3630-3635.
8. Maiti, C.K.; Sen; Paul, A.M. and Acharya, K., (2007) : First report of leaf blight disease of gloriosa superb L. caused by *Alternaria alternata (*Fr.) Keissler in India. Journal of General Plant Pathology 78 : 377-378.
9. Muhammad Bismillah khan, Tauqeer Ahmad and Madiha Aman, (2005): Growth and YIeld comparison of different linseed (*Linum usitatissimum L.*) Gonotype planted at different Row Spacing. *I.J. Agri, and Bio., 1566-8530, 3-515-517.*
10. Radhamani, J.; Dubey, S.P.; Srivastava, R.L. and Singh, A.K., (2006): genetic Resources of Linseed (*Linum usitatisimum L.*) conservation and citilization in crop imporvement. *Indian Jounral of Plant genetic resources* Vol. 19 : (1) : ISN 0971 – 8184.
11. Richharia, R.H., (1962): Linseed the India central Oil seed committee, Hyderabad, pp. 155.
12. Siddiqui, M.R. (1963): Taxonomy and pathoginicity of the genus *Alternaria* with special reference to *India species Alternaria tennuis J. Indian Bot.* Soc. 42 : 260- 272.
13. Singh Vivek and Singh Jyoti (2007): Evaluation of Plant Extracts against *Alternaria* Blight of Linseed (*Linum usitatissiumum* L.) Amals of Plant profection science Vol. 15 pp. 0971-3573.
14. Vincent, J. M.; (1947). Distortation of fungal hyphae in the presence of certain inhibitors. Nature. 159-850.
15. Zajac Tadeus, Stonislaw Grazesiak, Bogdan Kuling Milan polacek, (2005): The estimation of productivity and yield of linseed (*Linumusitatissimum* L.) using the growth analysis vol. 27 4 A pp 249 – 558.