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Evaluation of Different Botanicals Against Alternaria Blight Disease of Linseed (*Alternaria Lini*)

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***Abstract*: A study on evaluation of different botanicals against Alternaria blight disease of linseed was carried out in year 2018-19. *In vitro* study of leaf extract of eight local plants as *Azadirachta indica*, *Ocimum tenuiflorum*, *Lantana camara*, *Jatropha curcas*, *Melia azedarach*, *Datura stramonium*, *Calotropis procera* and *Citrus limon* was carried out (at 7.5% and 10% concentration) against Alternaria blight disease pathogen *Alternaria lini (A. lini)*. Results revealed that leaf extract of *Azadiracta indica* and *Ocimum tenuflorum* showed highest efficacy about 85.71% and 82.85% inhibition of the average mycelia growth of the *A. lini* at 10% and about 82.85% and 81.42% at 7.5% concentration. Whereas *Citrus lemon* was found least effective with 45.71% and 48.57% mycelial inhibition at 7.5% and 10% concentrations respectively. Therefore, *Azadiracta indica* and *Ocimum tenuflorum* can be treated as most useful, cost effective and eco-friendly natural fungicide to prevent Linseed crop from *A. lini* infection.**

***Keywords*: Azadirachta indica, Botanicals, Concentrations, Growth inhibition.**

1. Introduction

Linseed (*Linum usitalissimum* L.) grown in different parts of India as oil seed and fibre crop. Linseed has various industrial, medicinal and food values. Linseed consist α- linolenic acid (ω-3 fatty acid), protein and soluble mucilage. Linseed oil possess ω-3 (57%), ω-6 (16%), monosaturated fatty acid (18%) and saturated fatty acid (9%) in its composition [Katare et. al 2012] [1]. Healthy components present in Linseed draws the attention of food technologists and nutritionists to investigate its activities and utility in public health sector [Mishra and Verma, 2013] [2]. Researchers proved that, ω-3 fatty acid reduced the risk of cardiovascular disease [Hurteau MC. 2004] [3]. Protein isolated from Linseed was found more effective in lowering plasma cholesterol and triglycerides (TAG) as compared to soy protein and casein protein [Bhathena] [4]. The antioxidant activity of the Linseed has been also reported to reduce total cholesterol and platelet aggregation [Bierenbaum et al, 1993; [5] Allman et. al, 1995] [6]. It is also economically important crop and generally used for oil and fibre isolation. These features of Linseed, attracts farmers worldwide for its cultivation at large scale. India having second position in area (437 lac hactares) and forth position in Linseed production (1.68 lac tonnes) with an average productivity of 449 kg/ha [Srivastava 2010] [7]. But this production is less than the average production of Asia (575 kg/ha) and world (867 kg/ha) [Anonymous 2010] [8], while India having a huge farming land. There are so many reasons of its lower productivity in India, from which diseases are the main reason due to which farmers faced many problems to grow Linseed. Alternaria blight is most common disease of Linseed caused by a specific pathogen *Alternaria lini (A. lini*). This disease affects the aerial parts as leaves and buds of infected plant and in severe condition the seeds of plant can also contaminate. This disease leads to a heavy loss in quality as well as in quantity of fibre and seed of Linseed. Alternaria blight of Linseed was firstly reported by Dey [1933] [9]. Later Arya and Prasad (1952) [10] and Siddiqui (1963) [11] also reported the occurrence of Alternaria blight on linseed caused by *A. lini*. Sharma et al, 2015 [12], reported that the infection of *Alternaria* blight disease in Linseed was usually occurred during its flowering stage, under field conditions of Bundelkhand. Farmers are usually used some chemical fungicides as carbendazim etc. to prevent their crop from A. lini. The use of synthetic chemicals is not only costly but also produce environmental hazards and health risks to living beings, due to their toxicity and environmental persistency. Therefore, it is necessary to diminish the use of hazardous chemicals for controlling Alternaria blight disease. Researchers are continuously working to find out some economic and ecofriendly ways to control *A. lini* infection in Linseed. For this purpose, role of physiological and environmental implications as change in sowing dates [Singh et al, 2015] [13], alteration in sowing medium temperature and soil pH have been demonstrated to control growth of *A. lini* [Kumar et al, 2018] [14]. Integration of plats products and biocontrol agents with fungicides has been reported earlier [Singh et al. 2014] [15]. Currently researchers are emphasising on use of simply plant products (without incorporation of fungicides) to inhibit growth and development of *A. lini*, which is the most convenient, cost effective and eco-friendly way for crop prevention from alterneria blight. Plant products are easily approachable,

disposable and biodegradable. In this view, the attempt has been made to determine the efficacies of leaf extracts of eight botanicals belongs to different families, against *A. lini* causal organism of blight disease of linseed, under *in vitro* condition. For this study, two different concentrations i.e. 7.5% and 10% have been taken.

2. Materials and Methods

A. Collection of fungi

Leaves and buds of linseed showing the characteristics symptoms were collected from agriculture fields of Jhansi. These collected plant parts were sterilized thoroughly with alcohol and were brought to laboratory for isolation of disease causal pathogen (*A. lini*).

B. Isolation of the pathogen

Infected leaves and buds of linseed were firstly washed with triple distilled water. Cross section of lesion containing infected tissues was cut of up to 5 to 10 mm square. Surface sterilization procedure was followed by sterilization of the cut portions with 0.1% mercuric chloride ($MgCl_2$) solution for 30 seconds. Treated infected tissues were further washed with sterile water and then air dried. Finally, the infected tissues were transferred into petri plates containing Potato Dextrose Agar (PDA) media and placed in biological oxygen demand (BOD) incubator for inoculation. After 2-3 days, whitish mycelia growth was appeared around the infected tissue. The hyphal tips of mycelium were transferred in PDA culture tubes. After microscopic examination of obtained culture, presence of pathogen (*A. lini*), responsible for blight disease development (in collected plant parts) was observed.

C. In vitro evaluation of botanicals against blight disease of linseed

Under *in vitro* conditions, eight local botanicals belong to different families as *Azadirachta indica* (neem), *Ocimum tenuflorum (*tulsi*)*, *Lantana camara (*lantana*)*, *Jatropha curcas (*jatropha*)*, *Melia azedarach (*drake*)*, *Datura stramonium (*datura*)*, *Calotropis procera (*aak*)* and *Citrus limon (*lemon*)* were evaluated against *Alternaria lini* using PDA as basal medium. Fresh leaves of above local botanicals were separately washed with distilled water, then dried and crushed by pestle-mortar eventually filtered through muslin cloth. This filtrate contains 100% plant extract solution. The plant extracts were sterilized in autoclave for 20 minutes, at 121°C and 15Ib/in². Using sterile pipette, 7.5% and 10% concentration of leave extracts added separately in eight petri plates containing PDA medium. Eight circular discs of 5mm circles were cut by cork borer from 7 days old culture of pathogen *A. lini* and then placed separately on each petri plate containing different plant extract. One petri plate of PDA medium (without plant extract) inoculated as control. Three replications were made for each treatment and untreated control petri plate incubated at 25±2°C in BOD incubator. After incubation of 7 days, the diameter of mycelial growth was measured in each treatment and control plates. The efficacy of each plant extract was observed and determined against growth of pathogen in untreated control plate. The mycelial growth of pathogen *A. lini* was recorded on the tenth day when untreated control plates were observed to have maximum growth. Three replicates of the same experiments were performed. The percent inhibition was calculated using the following formula (Vincent, 1947) [16].

$$I = \frac{C - T}{C} X\ 100$$

Here I is percent inhibition of mycelial growth, C denotes for radial growth of fungus in control and T is radial growth of fungus in treatment.

3. Statistical analysis

Statistical analysis was done with using the standard procedure described by Gomez and Gomez (1986) [17].

4. Result and discussion

A. Identification of pathogen

Identification of isolated pathogen was carried out on the basis of its morphological characteristics. This study revealed that the morphological characteristics of isolated pathogen were same as reported by Dey (1933) [9] for *Alternaria lini*.

B. Study on efficacy of botanical extracts against A. lini

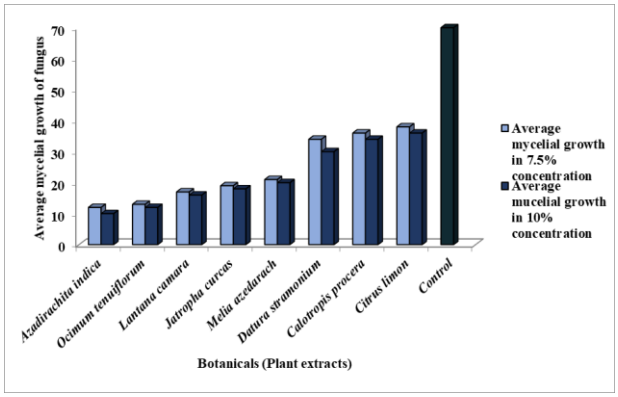
In vitro evaluation of eight botanicals against *A. lini* using PDA as medium was carried out. Data mentioned in Table 1. Results suggested that at 7.5% concentration (of plant extracts), *Azadirachta indica* followed by *Ocimum tenuflorum* both have been showed significant activity against *A. lini* with minimum mycelial growth (12 mm and 13 mm respectively) and maximum inhibition percentage (82.85% and 81.42% respectively). The order of activity of other botanicals at the same concentration is as follows: *Lantana camara* (17 mm growth and 75.71% inhibition), *Jatropha curcas (*19 mm growth and 72.85% inhibition), *Melia azedarach* (21 mm growth and 70% inhibition), *Datura stramonium* (34 mm growth and 51.42% inhibition), *Calotropis procera (*36 mm and 48.57% inhibition) and *Citrus limon* (38 mm growth and 45.71% inhibition). So, highest mycelial growth and minimum inhibition was shown by *Citrus limon* (results depicted in graph 1 and 2). Further the activity of leaf extracts of same botanicals was also checked at higher concentration to observe the effect of concentration on their activity. In order to find out the efficacy of above botanicals at higher concentration (of extracts), the similar experiments were repeated with 10% concentration of leaf extracts of each botanical. Data has been shown in Table 1. Pictorial representation of experiments has been shown in Figure 2.

From Table 1, it is clear that the inhibition of *A. lini* by used botanicals was enhanced after enhancement of concentration of leaf extracts. Results suggested that the enhancement in leaf extracts concentration from 7.5 % to 10 % positively affects the

Table 1
In vitro evaluation of botanicals against *A. lini.*

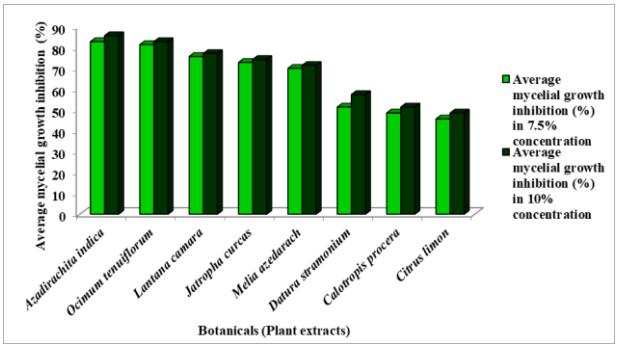
Botanicals	Family	Average mycelial growth (mm)		Mean	Average mycelial growth inhibition (%)		Mean
		Concentration (%)			Concentration (%)		
		7.5	10		7.5	10	
Azadirachta indica (Neem)	Meliaceae	12	10	11	82.85	85.71	84.28
Ocimum tenuiflorum (Tulsi).	Lamiaceae	13	12	12.5	81.42	82.85	82.13
*Lantana camara (*Lantana*)*	Verbenaceace	17	16	16.5	75.71	77.14	76.42
*Jatropha curcas (*Jatropha*)*	Euphorbiaceace	19	18	18.5	72.85	74.28	73.56
*Melia azedarach (*Drake*)*	Meliaceae	21	20	20.5	70	71.42	70.71
*Datura stramonium (*Datura*)*	Solanaceace	34	30	32	51.42	57.4	54.41
*Calotropis procera (*Aak*)*	Asclepidaceace	36	34	35	48.57	51.42	49.99
*Citrus limon (*Lemon*)*	Rutaceae	38	36	37	45.71	48.57	47.14
Control		70	70		-	-	
Mean		23.75	22		66.06	68.59	

activity of these botanicals towards *A. lini*. A significant hike in inhibition of *A. lini* by above botanicals was observed at 10 % concentration (Graph 1, 2 and Figure 1). At higher concentration (10 %) again *Azadirachta indica* and *Ocimum tenuiflorum* found as most effective botanicals with minimum mycelial growth (10 mm and 12 mm respectively) and maximum inhibition (85.71 % and 82.85 % respectively). The order of activity of other botanicals was found as same as in 10 % concentration (Table 1, graph 1 and 2). *Citrus limon* founds least active against *A. lini* with 48.57 % inhibition and 36 mm mycelial growth as compared to control (70 mm mycelial growth). Result indicated that leaf extracts of *Azadirachta indica* was found most effective at both concentrations (7.5 % and 10 %), it has been shown excellent inhibition of mycelial growth of *A.lini*, causal organism of Alternaria blight disease of linseed. *Ocimum tenuiflorum* also found good against this pathogen. So we can conclude that the leaves of *Azadirachta indica* and *Ocimum tenuiflorum* both can be useful to control blight disease caused by A. lini.



Graph-1: *In vitro* effect of botanicals (plant extracts) on the growth of *A. lini.*

The present results are in parellal with previous botanical extracts based pathogen inhibition studies. Leaf extracts of *Azadirachta indica* and *Ocimum tenuiflorum* were found most effective against blight causing pathogen. On the other hand, extracts of *Lantana camara*, *Jatropha curcas*, *Melia azedarach*, *Datura stramonium* and *Calotropis procera* also possess ≥ 50 % inhibition at 10 % concentration. Efficacy of *Azadirachta indica* (leaves) against some soil borne pathogens has been previously reported by Singh et al (1980) [18]. Aqueous leaf extract of *Azadirachta indica* was previously reported for inhibition of *A. lini* the causal organism of leaf and bud blight in linseed [Singh and Singh, (2007)] [19]. Leaf extract of *Azadirachta indica, Ocimum tenuiflorum, Datura stramonium, Vinca rosea, Polylthia longifolia* have been also reported to possess activity (at 1000 µg/ml) against many pathogenic fungi (Shivpuri et al 1997) [20]. Bansal and Gupta (2000) [21] reported the efficacy of extract of *Azadirachta indica, Lantana camara* and *Ocimum basilicum* (at 100% concentration) for the inhibition of fenugreek wilt pathogen *Fusarium oxysporum*. Plant extract of *Azadirachta indica* and *Jatropha curcas* were also found effective (at 6% concentration) against soil phytopathogenic and mycotoxigenic fungi *Fusarium oxysporum*, *Alternaria alternata* and *Aspergillus flavus.*



Graph-2: *In vitro* effect of botanicals (plant extracts) on mycelial growth inhibition of *A.lini.*

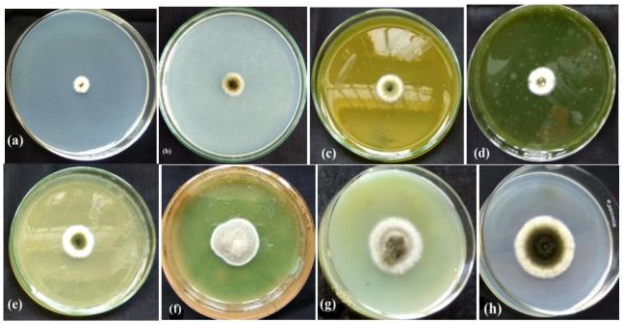


Fig. 1. *In vitro* effect of different botanicals (a: *Azadirachta indica*, b: *Ocimum tenuiflorum*, c: *Lantana camara*, d: *Melia azedarach*, e: *Jatropha curcas*, f: *Datura stramonium*, g: *Calotropis procera*, h: *Citrus limon* on mycelial growth inhibition against *A. lini.* (in 10% concentration)

5. Conclusion

Alternaria lini, causal pathogen of blight disease in Linseed was isolated and identified. PDA used as a growth medium for isolate the pathogen (*A. lini*) form infected portion of samples. Eight local plant extract belongs to different family, *in vitro*, tested against *A. lini* at two different concentrations (7.5% and 10 %). Results revealed that extract of *Azadirachta indica* and *Ocimum tenuiflorum* showed good potential for the inhibition of *A. lini*. Moreover, results also suggested that the activity of extracts against pathogen was enhanced significantly on enhancement of concentration. The order of activity was found as *Azadirachta indica* >*Ocimum tenuiflorum* >*Lantana camara* > *Jatropha curcas* > *Melia azedarach* > *Datura stramonium* >*Calotropis procera* >*Citrus limon*. Lowest inhibition of *A. lini* was shown by *Citrus limon.* From the results it can be concluded that leaves of *Azadirachta indica* and *Ocimum tenuiflorum* can be useful to prevent the Linseed crop from blight disease by controlling the growth and development of *A. lini* on it. Moreover, the use of these botanicals can also be helpful for the cost effective and eco-friendly management of Linseed farming.

References

[1] C. Katare, S. Saxena, and S. Agrawal, GBKS Prasad, P.S. Bisen, "Flax Seed: A Potential Medicinal Food", *J Nutr. Food Sci.*, vol. 2, no.1, pp. 120-128, 2012.

[2] S. Mishra, and P. Verma, "Flaxseed- Bioactive compounds and health significance", *IOSR Journal of Humanities and Social Science*, vol. 17, no. 3, pp. 46-50, 2013.

[3] M.C. Hurteau, "Unique new food products contain good omega fats", *Journal of Food Science Education*, vol. 3, no. 4, pp. 52-53, 2004.

[4] S.J. Bhathena, A.A. Ali, Mohamed A.I., C.T. Hansen, and M.T. Velasquez, "Differential effects of dietary flaxseed protein and soy protein on plasma triglyceride and uric acid levels in animal models", *Journal of Nutritional Biochemistry*, vol. 13, no. 11, pp. 684-689, 2002.

[5] M.L. Bierenbaum, R. Reichstein, and T.R. Watkins, "Reducing atherogenic risk in hyperlipemic humans with flax seed supplementation-A preliminary report", *J. Am. Coll. Nutr.*, vol. 12, pp. 501-504, 1993.

[6] M.A. Allman, M.M. Pena, D. Pang, "Supplementation with flaxseed oil versus sunflowerseed oil in healthy young men consuming a low fat diet: effects on platelet composition and function", *Eur. J Clin. Nutr*, vol. 49, pp. 169-178, 1995.

[7] R. L. Srivastava, "Linseed: technology for increased production", 3rd ed., *Chandra shekhar azad university of agriculture and technology*, Kanpur, India 2010

[8] Anonymous, "annual report Linseed: all india coordinated research project on Linseed, project coordinating unit", *Chandra shekhar azad university of agriculture and technology*, Kanpur, India, 2010.

[9] P.K. Dey, "An Alternaria blight of linseed of plant", *Indian J Agri. Sci.*, vol. 111, pp. 881-896, 1993.

[10] H.C. Arya, R. Prasada, "Alternaria blight of Linseed", *Indian Phytopath*, vol. 5, pp. 33-39, 1952.

[11] M.R. Siddiqui, "Taxonomy and pathoginicity of the genus *Alternaria* with special reference to Indian species *Alternaria tenui", J. Indian Bot. Society*, vol. 42, pp. 260-272, 1963.

[12] R.B. Sharma, A. Arjariya, R. Singh, "Bight Disease of Linseed with its effects on oil quality and ethnomedicinal control", *Periodic Research*, vol. 4, no. 2, pp. 22-26, 2015.

[13] V. Singh, M. Lal, S. Kumar, Mohd. Ali, J. Singh, "Management of alternaria blight of linseed with sowing dates and host resistance", *Universe of Emerging Technologies and Science*, vol. 2, no. 4, pp. 1-4, 2015.

[14] S. Kumar, A. K. Meena, P. N. Meena, "Effect of Different Physiological Parameters on the Growth of *Alternaria cucumerina* var. *cyamopsidis* (Rang. and Rao)", *International Journal of Current Microbiology and Applied Sciences*, vol. 7, no. 9, pp. 2993-2998, 2018.

[15] R.B. Singh, H.K Singh, A. Parmar, "yield loss assessment due to alternaria blight and its management in Linseed", *Pakistani Journal of Biological Sciences*, vol. 17, no. 4, pp. 511-516, 2014.

[16] J.M. Vincent, "Distortion of fungal hyphae in the presence of certain inhibitors", *Nature.*, vol. 159, pp. 850-853, 1947.

[17] K.A. Gomez, A.A. Gomez, "Statistical procedures for agriculture research", 2nd ed., *John Wiley and Sons*, pp. 680, 1986.

[18] U. P. Singh, H. B. Singh, R. B. Singh, "The Fungicidal Effect of Neem (*Azadirachta indica*) Extracts on Some Soil-Borne Pathogens of Gram (*Cicer arietinum*)", *Mycologia*, vol. 72, no. 6, pp. 1077-1093, 1980.

[19] V. Singh, J. Singh, "Evaluation of plant extracts against *Alternaria* blight of linseed *(Linum usitatissimum* L.)" *Annuals of plant protection science*, vol. 15, pp. 0971-3573, 2007.

[20] A. Shivpuri, O. P. Sharma, S. L. Jhamaria, "fungitoxic properties of plant extracts against pathogenic fungi", *journal of mycology and plant pathology*, vol. 27 no. 1, pp. 29-31, 1997.

[21] R.K. Bansal, R.K. Gupta, "Evaluation of plant extracts against Fusarium oxysporum, wilt pathogen of fenugreek", *Indian Phytopathology*, vol. 53, pp. 107-108, 2000.