Research Paper
Impact Factor: 6.089
Peer Reviewed Monthly Journal
www.ijmdrr.com

IJMDRR
E- ISSN –2395-1885
ISSN -2395-1877

EFFECT OF NEEM LEAF EXTRACT (*AZADIRACTA INDICA* (A).JUSS) AND NEEM PRODUCTS ON CONIDIAL GERMINATION OF SELECTED FUNGAL PATHOGENS OF SORGHUM (*S.BICOLAR.* L).

Dr.R.S.Kallurmath
Associate Professor and Head the Department of Botany,Government. First Grade College for Women, Bijapur. Karnataka.

Abstract
Studies were undertaken to know the effect of different concentrations of Neem leaf extract (Azadiracta indica.A .Juss) and Neem products (Nimebicidine, Neem azal and Neemark), on the conidial germination of selected fungal pathogens viz., Alternaria alternate, Claviceps fusiformis, Colletotrichum graminicola, and Phyllactina corylea, of Sorghum (S.bicolar..L) Variety M.35-1.The neem leaf extract and neem products had no adverse effect either on seed germination or seedling vigor and different concentrations of study source indicates, that they inhibites the conidial germination selective fungal pathogens were determined.

Key Words: Neem s (Azadiracta indica), Conidia, Suspension, Fungicide, Antifungal activity. Neem products, Serial dilution.

Introduction

The survey of literature reveals that a good account of research was done on insecticidal, pesticides and nematicidal activities of neem. However the fungicidal activities were less documented. In the present study attempts were made find the effect of neem leaf extract (aqueous) and neem products available commercially on few important selectives fungal pathogens of Sorghum.

Neem (*Azadiracta indica*.(A).Juss) is a medium sized tree and belongs to a member of the family Meliaceae and native of South Asia, also found in parts of South East Asia and Africa. It is cultivated all over the India, Chaina, and Burma. It performs better than many other fast growing species in arid and semiarid regions and lives about 200 years. Neem grows on all kinds of soils ,it tolerates soil salinity, alkalinity, and acidic condition and also establishing well even in severe drought prone areas and grows as a avenue tree (Ezumah, 1986)

Neem as a Fungicide

Studies have been conducted in India and elsewhere and established (Kerni., et al 1983) the effects of various vegetables and using different plant parts of neem and tulsi in controlling the diseases like blue mold ,Erisiphe,Powdery mildew, colletotrichum, and others(Singh., and Chouhan.1984) of Pea, Cotton, Banana , Jawar, etc., The extracts of different parts of neem plant concentrations have been studied, the observation on spore density and germination were made with control sets .The density of pathogen inoculums was greatly reduced. The inhibitory effect of neem leaf extract on aflotoxin synthesis in *Aspergillus parasiticus* was observed by many researchers (Bhatnagar and Deepak, 1988. Zeringue,et.al 1990). Neem leaf extracts of different dilutions concentrations shows an aflotoxin inhibitory factor.The effects of oil cakes of Neem and Castor were studied (Rahaman ,et.al 1988.) on seed germination of weed called *Chenopodium album* suppressed the fungal growth. Studies on wood ashes of *Azadiracta indica* were tested against seed borne fungi in Maize and significant reduction in density of fungal spores that may affects on seed germination and pre-mature mortality was recorded. Also attempts were made to manage damping off of tomato, chilli, and brinjol along with synthetic neem products and was found in greater reduction in incidence of the diseases. Neem and its parts are used in pest control of various millets since long back traditionally and even now a days farmers use neem leaves and are mixed in bags of sorghum, wheat, bajra in storing of seeds and other grains to avoid pests from decompose. In the present study attempts have been made to know the antifungal effects neem leaf extract (aqueous) and commercially available neem products and compared their efficacy against selected fungi.

Materials and Methods

Isolation and culturing of pathogens from host

Infected parts of Sorghum plant (leaf, stem, inflorescence and seeds) were collected from the sorghum fields and brought to the laboratory for father study and the fungal pathogens were isolated on Potato Dextrose Agar (PDA) medium under sterilized conditions. Standered methods were followed in sterilizations of glass wares and storing the medium (Tuite.,J 1960) . The stored PDA medium was sterilized and semi solid media was poured to the sterilized Petriplates . 1ml of streptomycine was added to culture medium to check the bacterial contamination. When the medium solidifies the conidia of test pathogens (*Alternaria alternate, Claviceps fusiformis, Colletotrichum graminicola, ,* and *Phyllactina corylea*.) were inoculated on to the medium carefully with sterilized needles under controlled conditions separately. The inoculated Petri plates were kept for incubation under sterile laboratory condition.

Observations

The incubated Petri plates were observed on fifth day after incubation and colony forming units of test fungi were further identified using available literature (Barnett and Hunter, 1990) ,and are used in further study whenever necessary pure isolates of test fungal pathogens were sub cultured for feature use.The conidia from pure cultures of isolates were used in germination studies to screen the antifungal properties of neem and neem products.

Preparation of neem leaf extract:

1. 100 gms of fresh neem leaves collected from the field were washed thoughrolly and crushed in 100 ml of distil water till to get fine slurry. The extract thus obtained was filtered through muslin cloth and centrifuged for 15 minutes at 1000 rpm. ,the precipitate was discarded and supernant of the extract was considered as 100% stock solution. From this extract a number of serial dilutions (5%, 10%, 15%, 20% and 25% concentrations) were prepared by adding distilled water.

2. **Preparation of different concentrations (5% to 25%) of neem products (Neemazal, Nimbecidine, Neemark) solutions:**
   Samples of NeemAzal , Nimbecidine, and Neemark supplied by M/S Stanny and Parry (Indi) Ltd., companies respectively was used in the present study. 3 ml of test solutions was pipette out in to three separate conical flasks and each of the products was dissolved in 1000 ml of distilled water. The solution was considered as 100% stock solution. From this solution different concentrations (5%, 10%, 15%, 20% and 25%) of solution were prepared using distilled water.

3. **Conidial germination of test pathogens and effect of neem and neem products:**
   i). Preparation of conidial suspension:
   The parts of sorghum plants showing disease symptoms were collected from the fields and are thoroughly washed with distilled water. The diseased parts of plant were cut in to small pieces and were kept in sterilized petriplates lined with moist blotter for 48 hours for sporulation.
   After 48 hrs of incubation, the conidia were collected and conidial suspension were prepared using distilled water, such prepared spore suspension was used in study.

   ii). Conidial germination
   The effect of Neem Azal. Nembecidine , and Neemark and Neem leaf extract of prepared concentrations on conidial germination of *Alternaria alternate, Claviceps fusiformis, Colletotrichum graminicola,* and *Phyllactina corylea,* was determined by employing moist blotter method.A drop of neem leaf extract (selected concentrations) was put on a cavity slide containing a drop of conidial suspension of test pathogen .With the help of sterile needle, the two solutions (conidial suspension and neem leaf extract) were mixed thoroughly. The same method was followed with regard to neem products and set of distilled water with test pathogen served as control.

Prepared cavity slides were kept in petriplates lined with moist blotters. The slides were positioned on two horizontally placed glass rods up to 24 hours at 25 0 C. The slides were taken out from Petri plates and mounted using cover glass of size 22X50 mm and a drop cotton blue was added. Excess solution was drained off using blotting paper.

The slides were observed under microscope to record the conidial germination. The data were expressed as per cent conidial germination, using following formulae

$$\% \text{ conidial germination} = \frac{\text{Total no. of conidia germinated}}{\text{Total no. of conidia observed}} \times 100$$

4.Effect of Neem leaf extract and Neem products on germination of Sorghum seeds:
Sorghum seeds were collected from local Agricultural research station, and are washed thoroughly in distilled water and soaked in 100% concentration of neem leaf extract (100 gms of fresh neem leaves crushed in 100 ml of distilled water) Nimbecidine, (3 ml dissaived in 1000 ml of distilled water) ,and similar procedure was followed with NeemAzal, and Neemark.

Such prepared seeds were observed after 24 hours , 60 such seeds were selected from each treatment and placed in Petri plates lined with moist blotter separately for each treatment. The seeds soaked in distilled water were also placed in petriplate lined with moist blotter, which served as control. After 3 days of incubation, observations were made and recorded the data in per cent germination of seeds by taking in to the consideration of the length of plumule and radical of each treatment.

Results and Discussion
Studies were undertaken to know the effect of different concentrations of neem leaf extract and commercial neem products (Nimbecidine, Neem Azal, and Neemark), on conidial germination of selected fungi, viz., *Alternaria alternate, Claviceps fusiformis, Colletotrichum graminicola,* and *Phyllactina corylea,* which are common pathogens causing diseases in sorghum, leads to less in yield, market value, consumers rejection and other factor makes formers in to monitory and storage loss. (Gandhi. 1988). Experimental results are presented to each fungal pathogens in separate tables.

Alternaria alternata :
The sorghum seeds were incubated in moist blotters, some of the seeds showed the colonization by *A.alternata*. From such seeds the pure cultures of the fungus were isolated on PDA medium and conidia were used in the experimental study and presented in Table-1

The percent conidial germination in test solutions i.e in neem leaf extract and Nimbecidine varied from 47% to 87% and 56% to 83% respectively.,86% germination was observed in control. Whereas, in Neemazal , the per cent germination varied from 26% to 88% was observed in different concentrations (5% to 25%) . Table -1, further shows among the three treatment (NLE, Nimbecidine, and Neemazal) , Neem Azal was found to be more inhibitory activity to conidial germination. As the concentration increases the per cent of conidial germination decreased. The conidial germination was minimum at 25% (26%) followed by 20%(46%), 15% (84%), 10% (85%) and 5% (88%) . Whereas Neem leaf extract and Nimbicidine were found to inhibit the germination of conidia of *A.alternata* at lower concentrations. The per cent conidial germination at 5% concentration was less in Neem leaf extract (47%) and Nimbecidine (56%).Whereas, the conidial germination percentage was more in 25% concentration of neem leaf extract (87%) and Nimbecidine (83%).

Claviceps fusiformis:
The conidia of *C.fusiformis* were harvested from sorghum earheads at honey dew stage. Different concentrations of Neem leaf extrct, Nimbicidine , NeemAzal and Neemark were prepared and tested to know their inhibitory effect if any on the conidial germination of Ergot Pathogen.

The per cent conidial germination in neem leaf extract Nimbecidine varied from 62% to 72% and 63% to 79%, 85% conidial germination was observed in control experiments. Similarly, the germination in Neemazal and Neemark varied from 60% to 81% and 55% to 81% in different concentrations used (5% to 25%) . Maximum germination of conidia was found in 5% concentration of NeemAzal and Neemark (81%). Whereas, minimum conidial germination 55% was found in 25% concentration of Neemark. Table-2.

Among different experiments conducted (concentrations of 5% to 25%)., 25% of Neemark was found to inhibit the conidial germination (45%, inhibition) followed by 25% of NeemaAzal (40% inhibition). 20% neem leaf extract (38% inhibition) and 25% Nimbecidine(37% inhibition).

Colletotrichum graminicola:
Sorghum leaves showing anthracnose symptoms were brought to the laboratory and pure cultures of *C.graminicola* were isolated on PDA medium, the conidia were harvested and used in the present study to determine the inhibitory effect of neem leaf extract, Nimbecidine, Neem Azal, and Neemark on conidial germinaton.

The conidial germination was 11%, 11%, 10%, 9%, and 9% respectively in 5%, 10%, 15%, 20%, and 25% concentrations ,of neem leaf extract as compared to 81% conidial germination in control treatments. The conidial germination was 44% to 68% in Nimbecidine, 54% to 80% in Neem azal and 65% to 75% in Neemark. All the concentrations of neem leaf extract were highly inhibitory to the germination of conidia of *C.graminicola* as compared to control treatments.All the prepared concentrations of neem leaf extracts were equally effective in inhibition of conidial germination compared to commercial neem products. Table -3

Phyllactina corylea :
Along with neem leaf extract the neem products as a test solutions were studied on the effect of conidial germination of *P.corylea* .A double moist blotter method was used in the study, in which one blotter was lined with lower lid of the Petri plate and another was placed on the two glass slides and they are positioned over two horizontally placed glass rods in the Petri plate and moistened with distilled water along with control set.

A cellophane paper (18mm X 18mm) was placed on blotting paper and carefully dusted with conidia of test fungi.,such prepared petriplates were incubated at room temperature for 48 hrs. After incubation the cellophane strips were removed and mounted on clean microscopic slide

Using a drop of cotton blue stain, examined under microscope to record the total number of per cent of conidial germination including control set and calculated.

It was observed that all the concentrations of neem leaf extract and neem products inhibited the conidial germination in *P.corylea* as compared to control. (Table-4). The percent of conidial germination in neem leaf extract and neem products are varied from 20% to 46% and 12% to 38% as compared to 77% in control . Where as in Neem azal and Neemark the percent germination varied from 18% to 55% and 11% to 57% respectively. The maximum, conidial germination was observed in 5% of Neemark (57%) followed by Neemazal (55%) neem leaf extract (46%) and Nimbecidine (38%). Table-4

Conclusion
In general the percent of conidial germination decreased with increase in the concentrations of neem leaf extract and neem products. In all cases 25% concentrations of neem leaf extracts and neem products were found to be

more effective on inhibition of conidial germination. The antifungal activities of all the selected test solutions used in the study are effective against the Sorghum pathogens. The studies were conducted in India and elsewhere have successfully established the antifungal nature of neem (Murugesan. 1994: Makanjuol. 1984: Bhatnagar and Deepak. 1988,), however in the present study attempts were made to compare the commercial neem products available in market with natural neem and its parts have shown equal efficacy in minimizing the disease incidence in sorghum caused by *Alternaria alternate, Claviceps fusiformis, Colletotrichum graminicola*, and *Phyllactina corylea*, . (Verma and et.al. 1998: Subapriya and Nagini. 2005. Charmaine and et.al., 2005). Suggest that it is to be used in standered form of neem leaf, seeds, and bark extracts in the form of fungicide to manage the fungal diseases in sorghum, as it has no cost is required also convenient and advantage to the formers to avoid fungal association in many crop plants. Standard protocol of neem leaf extracts and other parts, is to be technically develop for proper usage in agricultural practice from different form sectors to minimize the cost effect to the formers.

Acknowledgement
The author thank to the Department of Collegiate Education, Govt. of Karnataka for the encouragement and support.

References

1. Barnett.H.L and B.B.Hunter. 1972. *Illustrated Genera of Imperfect Fungi.IIIrd .Edn. Burg.Pub.Minn.* **pp-241**
2. Bhatnagar. C and Deepak. 1988. The inhibitory effect of Neem(*A.indica*) leaf extracts on aflotoxin synthesis in *Aspergillus parasiticus*.J.*of Chem.Sc*.**667**: 1166-1168
3. Charmaine L loyed A.C. Menon. T., Umamaheshwari K.2005. Anticandidal activity of *Azadiracta indica. Res.Pap*:37 **(6):** 386-389 (Google scholar).
4. Ezumah.B.S. 1986. Germination and storage of neem (*A.indfica*) *seed. Seed .Sci. Tech.*14**(3):**593-600
5. Gandhi. 1988. Acute toxicity of study of the oil from *A.indica seed.J of Ethnophamacolgy*.23(**1**):30-52).
6. Kerni.P.N,Shant.P.S and D.Singh. 1983. Effect of various vegetable oils in controlling blue mold (Penicillium expansum) rot ofd apple.*Prog.Hort.*15(**1/2**): 129-131
7. Makanjuola. W.A. 1989. Evaluation of extracts of neem (*A. indica*) for the control of some stored product pests. *J.of Stored Prd.Res*. 25(**4)**:231-238.
8. Murugesan. K. 1994. Antifungal activity of neemgold against to soil borne plant pathogenic fungi. *Geobios* (Jodhpur) 21(**3**): 173-176
9. Rahaman.R.,. and Wajid Khan. 1988. Efect of oil cake amendment on seed germination of Chenopodium album and population of fungi and nematodes. *Act.Bot.Ind*.16**(1**): 106-110
10. Singh.V.P.H. Singh .B and Chauhan..V.B. 1984.Effect of some fungicides ,plant extracts and an oil on inoculums density of different nodal leaves of Pea(Pisu sativum) infected by Erysiphae polygoni. *ZPflan.* 91(**1**): 20-26.
11. Subapriya.R and Nagini.S.2005. Medicinal properties of neem leaves.a.review.*Cur.Med.Chem.Anticancer agent*.5**(2**):149-160
12. Tuite. J. 1969. *Plant pathological methods.Fungi and Bacteria. Burg.Pub*.Minn**. pp-239**
13. Verma .D.K. Tripati.V.J. Rana.B.K. 1998. Antifungal activityof the seed coat extract of *A.indica.Ind.J.of Pharm .Sc.* 60(**5)**: 305-306.
14. Zeringue. 1990. Inhibitiopn of aflotoxins production in *Aspergillus flavus* in fected cotton balls after treaetment with neem leaf extract (*A.indica). Jam oil Chem. Soci*.34(**1**): 1-6.

Table. 1. Effect of Neem leaf extract and Neem products (Nimbecidine and NeemAzal) on the conidial germination of Alternaria alternate

Treatment	% of conidial germination in different concentration				
	5%	10%	15%	20%	25%
Neem leaf Extract.	47	68	84	84	87
Nimbecidine	56	66	58	81	83
Neem Azal	88	85	84	46	26
Control	86	86	86	86	86

Table. 2. Effect of Neem leaf extract and Neem products (Nimbecidine Neemark and NeemAzal) on the conidial germination of *C.fusiformis* conidia

Treatment	% of conidial germination in different concentration				
	5%	10%	15%	20%	25%
Neem leaf Extract.	75	72	65	62	62
Nimbecidine	79	78	75	67	63
Neem Azal	81	78	68	68	60
Neemark	81	67	66	65	55
Control	85	85	85	85	85

Table. 3. Effect of Neem leaf extract and Neem products (Nimbecidine Neemark and NeemAzal) on the conidial germination of *.Cgraminicola* conidia

Treatment	% of conidial germination in different concentration				
	5%	10%	15%	20%	25%
Neem leaf Extract.	11	11	10	09	09
Nimbecidine	68	67	55	52	44
Neem Azal	80	80	66	55	54
Neemark	75	75	74	73	65
Control	81	81	81	81	81

Table. 4. Effect of Neem leaf extract and Neem products (Nimbecidine Neemark and NeemAzal) on the conidial germination of *.Phyllactina corylea* conidia

Treatment	% of conidial germination in different concentration				
	5%	10%	15%	20%	25%
Neem leaf Extract.	46	27	24	23	20
Nimbecidine	38	36	34	24	12
Neem Azal	55	33	25	21	18
Neemark	57	24	23	14	11
Control	77	77	77	77	77