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# STUDIES ON THE SEED MYCOFLORA OF LENTIL AND ITS CONTROL

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Using the blotter technique 21 fungal species of fungi were isolated from 4 cultivars of lentil, of these 8 species are pathogenic on various crops. *Fusarium moniliforme, F. oxysporum, F. semitectum* were recorded in abundance. Treatments of seeds with 80 ppm neem bitter yielded significant control of seedborne mycoflora.

***Key words:*** Lentil, Seed mycoflora, Control, Neem bitter.

## Introduction

Lentil (*Lens culinaris* Medic.) is an important winter pulse crop in Pakistan. The crop is reported to suffer upto 20-25% losses in yield due to different diseases such as blight (*Ascochyta lentis* Bond and Vass), wilt (*Fusarium oxysporum* Schlecht), root rot (*Macrophomina phaseolina* (Tassi Goid), collar rot (*Sclerotium rolfsii* Sacc.), lentil rust (*Uromyces fabae* (Grev) de Bary, gray mold (*Botrytis cinerea* Pers.) and anthracnose (*Colletotrichum trifolii* Bain and Essary) [6,7,9,10,12].

The present studies were conducted to evaluate the effect of neem bitter on the mycoflora of lentil seeds. The neem plant has been historically popular for its insecticidal and medicinal activities. Several workers had shown the positive effect of neem against different pests, and it is presumed that neem products have no harmful effects.

## Material and Methods

Seeds of 3 lentil cultivars *viz.*, LPYTE2 77136, LPYTE2 77240 and LPYTE1 76147 were obtained from Pulses Programme, NARC, Islamabad. In addition, one cultivar was also taken from the local market. Blotter method as described by ISTA [2], was used to detect the seed mycoflora of lentil.

Effect of neem bitter on the seed mycoflora was also evaluated. Four hundred seeds of each cultivar were soaked in 80 ppm neem bitter solution for 30 min. and then placed on moistened blotters in 9 cm Petri dishes. A similar set of Non-treated seed used as control. The Petri dishes were incubated at 22° (±1) under 12 hr. diurnal cycles of light and darkness supplied by white fluorescent tube. The isolated fungi were identified to the species level after reference to Barnett and Hunter [3]; Ellis [5]; Nelson *et al* [8]; and Raper and Fennel [11].

*Preparation of Neem bitters.* Neem seed kernel was powdered, wrapped in a piece of muslin cloth and soaked for 12-16 hr., at room temperature (23°) in water with a pinch of detergent (Surf). The soaked material in muslin cloth was hand pressed to separate the liquid portion. The water extract was filtered through Whatman No. 1 filter paper, the filtrate was poured into a glazed tray and freeze-dried. The freeze-dryer was set at an initial shelf temperature of -40° and under a vacuum of 0.01 torr at -10°. Cooling was set at trap- temperature of 40°. The freeze drying process yielded a crystalline powder termed as Neem bitter.

## Results and Discussion

Using the blotter technique, 21 fungal species were isolated from the 4 cultivars of lentil used in this study (Table 1). Of the isolated fungi *Alternaria alternata* (Fr.) Keissler, *Fusarium moniliforme* Sheldon, *F. oxysporum* Schlecht, *F. semitectum* Berk. and Rev., *Curvularia lunata* (Wakker) Boedijn, *Cladosporium cladosporioides* (Frsen) de Vries, *Rhizoctonia solani* Kuhn and *Ascochyta* sp., are known to be pathogenic whereas *Aspergillus niger* Van Teigh., *A. flavus* Link, *A. terreus* Thom., *Chaetomium bostrychodes* Zopf., *C. globosum* Kunz ex Fr., *C. olivaceum* Cooke and Ellis, *Drechslera australiensis* (Bugnicourt) Subram. and Jain ex Ellis, *D. hawaiiensis* (Bugnicourt) Subram. and Jain ex Ellis, *D. spicifera* Nelson, *Memnoniella echinata* (Riv.) Galloway, *Paecilomyces varioti* Bainier, *Stachybotrys atra* Corda and *Stemphylium* sp., are non-pathogenic. Some of the isolated fungi like *A. flavus, A. terreus, Fusarium* spp., *M. echinata*, and *S. atra* are potential mycotoxin producers. The cultivar LPYTE2 77136 yielded highest number of fungi (17)followed by local cultivar (14), LPYTE1 76147 (13) and LPYTE2 77240 (12) respectively (Table 1). Among the fungi encountered *Fusarium* spp., exhibited higher incidence percentages in each cultivar and in each treatment followed by *Aspergillus* spp., and *Alternaria alternata* (Table 1).

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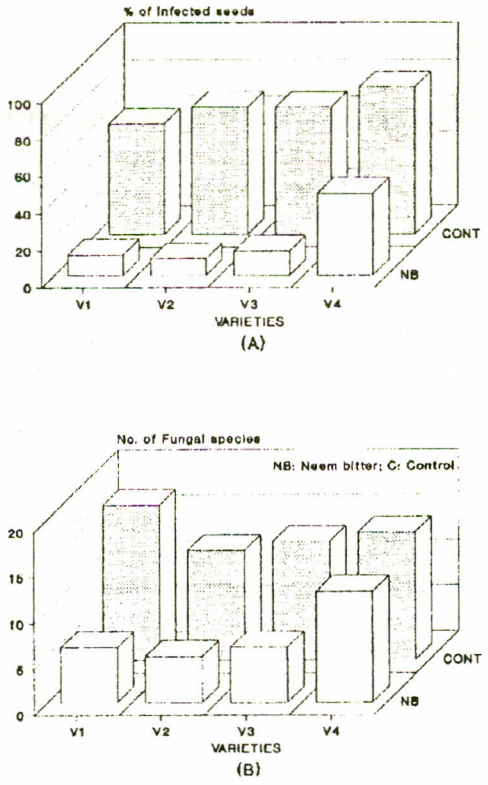
Data given in Fig. 1 and Table 1 shows significant differences among treatments in the control of seed mycoflora of lentil. Neem bitter yielded satisfactory control of seedborne mycoflora as the number of fungal species isolated from seeds treated with the neem bitter were comparatively less than those isolated from untreated seeds. Analysis of data using Two-way Analysis of Variance method [13], showed that the difference in number of species isolated was not significantly different between varieties, but difference between the treatments was significant at $P < 0.05$ level (Table 2). Similarly the difference in seed infection was not significant between cultivars, but was significant between the treatments at $P < 0.01$ level (Table 3).

Agarwal *et al* [1], and Suhag and Suryanraryana [12] found some control by the application of some fungicide especially in case of *Uromycesfabae* where Dithane M-45 was used @ 2500 ppm. Our results indicate that the effectiveness of Neem bitter in the control of seedborne fungi is comparable with that of fungicides as reported by Agarwal *et al* [1] and Suhag and Suryanraryana [12].

It would suggest that Neem bitter proved to be successful in controlling the seed mycoflora of lentil, though it was not efficient enough to give complete control of infection. It is being investigated whether higher concentrations and or a longer period of treatments would give better results.




$V_1$: LPYTE2 77136 $V_2$: LPYTE2 77240; $V_3$: LPYTE1 76147; $V_4$: Local cultivar.

Fig. 1. A: Percentage of infectd seeds in each treatment; B: Number of fungi recorded in each treatment.

Table 1. Effect of Neem Bitter on Seed Mycoflora of Lentil.

| Fungi isolated | $LPYTE_2$ 77136 | | $LPYTE_2$ 77240 | | $LPYTE_1$ 76147 | | Local | |
|---|---|---|---|---|---|---|---|---|
| | C | NB | C | NB | C | NB | C | NB |
| *Alternaria alternata* | 6 | 2 | 6 | 2 | 7 | 2 | 8 | 4 |
| *Ascochyta* sp. | – | – | 2 | – | – | – | – | – |
| *Aspergillus flavus* | 6 | – | 7 | – | 6 | – | 4 | 3 |
| *A. niger* | 8 | – | 7 | – | 6 | – | 5 | – |
| *A. terreus* | – | – | – | – | 3 | – | 2 | – |
| *Chaetomium bostrychodes* | 2 | – | – | – | 2 | – | – | – |
| *C. globossum* | 5 | 2 | 7 | 2 | 5 | 2 | 4 | 2 |
| *C. olivaceum* | 2 | – | – | – | – | – | – | – |
| *Cladosporium cladosporioides* | 4 | 2 | 4 | – | – | – | 6 | 4 |
| *Curvularia lunata* | 4 | – | 3 | – | – | – | 8 | 4 |
| *Drechslera australiensis* | – | – | – | – | 3 | 1 | 4 | 2 |
| *D. hawaiiensis* | – | – | 3 | 2 | 5 | – | 5 | 2 |
| *D. spicifera* | 3 | 1 | 4 | – | – | – | 4 | 3 |
| *Fusarium moniliforme* | 3 | 1 | – | 2 | 9 | 3 | 7 | 4 |
| *F. oxysporum* | 4 | 1 | 10 | 3 | 10 | 4 | 10 | 7 |
| *F. semitectum* | 3 | – | 9 | – | 7 | – | 10 | 6 |
| *Memnoniella echinata* | 3 | – | 3 | – | – | – | – | – |
| *Paecilomyces varioti* | 1 | – | – | – | – | – | – | – |
| *Rhizoctonia solani* | 2 | – | 4 | – | 2 | – | 3 | – |
| *Stachybotryts atra* | 2 | – | – | – | – | – | – | – |
| *Stemphyllium* sp. | 2 | – | – | – | – | – | – | – |

C: Control; NB: Neem bitter.

TABLE 2. ANOVA FOR THE % OF INFECTED SEEDS.

| Sources of variation | $d_f$ | Sum of squares | Mean squares | Computed f |
|---|---|---|---|---|
| Varieties | 3 | 920.5 | 306.5 | 6.84 ns |
| Treatments | 1 | 4900.5 | 4900.5 | 109.3* |
| Error | 3 | 134.5 | 44.83 | – |
| Total | 7 | 5955.5 | – | – |

* P< 0.01; ns: Non significant.

TABLE 3. ANOVA FOR THE NUMBER OF FUNGAL SPECIES.

| Sources of variation | $d_f$ | Sum of squares | Mean squares | Computed f |
|---|---|---|---|---|
| Varieties | 3 | 27 | 9 | 1.20 ns |
| Treatments | 1 | 98 | 98 | 14* |
| Error | 3 | 21 | 7 | – |
| Total | 7 | 146 | – | – |

* P < 0.01; ns: Non significant.

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