Efficacy of *Euphorbia splendens* and *Leonotis nepetaeJolia* on aflatoxin producing fungi *Aspergillus flavus* and *Aspergillus parasiticus*

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Efficacy of three different concentrations (5, 10 and 15 mg/ml) of dry flower powder of *E. splendens* and *L. nepetaeJolia* was tested on the growth of aflatoxin-producing toxigenic strains of fungi *A. flavus* (NCBT 101) and *A. parasiticus* (NCBT 128) in Sabouraud dextrose agar medium (SDA). Maximum (75%) inhibition of growth of *A. flavus* was seen at 15 mg/ml concentration of *E. splendens* flower dry powder, while *A. parasiticus* showed 50% inhibition of growth at 10 and 15 mg/ml concentrations. Total inhibition (100%) of growth of *A. flavus* was seen at 10 and 15 mg/ml for *L. nepetaeJolia* and maximum (75%) inhibition of growth was seen for *A. parasiticus* at 15 mg/ml concentration. Bioassay with groundnut seeds soaked with different concentrations of flower extract proved that both fungi were incapable of infecting the seeds at 10 and 15 mg/ml of *L. nepetaeJolia* flower extracts.

**Keywords**: Aflatoxin, *Aspergillus flavus*, *Aspergillus parasiticus*, *Euphorbia splendens*, *Leonotis nepetaeJolia*, Fungi

There are increasing number of reports on the presence of aflatoxins, secondary metabolites from fungi including *Aspergillus flavus* and *Aspergillus parasiticus* in foods and feeds, cause serious economic loss, apart from their carcinogenic potential. Many plant extracts have been used to study their antifungal properties and effects on mycotoxin production, and anti-aflatoxin properties. *Euphorbia splendens* (Euphorbiaceae) is a small perennial prickly much branched shrub with showy crimson flowers, and *Leonotis nepetaeJolia* (Lamiaceae) is a tall herb with orange-scarlet flowers. The effect of flowers of these plants as fungitoxicant on aflatoxin producing fungi *A. flavus* and *A. parasiticus* has not so far been studied. This study was undertaken to assess the fungitoxic role of the dry flower powder of *Euphorbia splendens* and *Leonotis nepetaeJolia* against the aflatoxigenic strain of *Aspergillus flavus* (NCBT 101) and *Aspergillus parasiticus* (NCBT 128). Flower aqueous extracts were also used for the bioassay of groundnut against these fungi.

The flowers of *Euphorbia splendens* and *Leonotis nepetaeJolia* were obtained from the College green house, washed initially with 2% aqueous NaOCl solution and subsequently with sterile distilled water. Flowers (20 g) were blended with 100 ml of sterile distilled water to prepare an aqueous extract. From this, mixture of different strengths (5, 10, and 15 mg/ml) was prepared and used for bioassay with groundnut seeds. The toxigenic strains of *Aspergillus flavus* (NCBT 101) and *Aspergillus parasiticus* (NCBT 128) maintained in the department were used in the present experiment. A 100, 200 and 300 mg of dry flower powder mixed with 20 ml of SDA medium (HI Media-M063) constitute 5, 10 and 15 mg/ml concentrations respectively. The control (i) contained only 20 ml of SDA medium; and control (ii) contained 100 mg of Bavistin fungicide added to 20 ml of SDA medium (5 mg/ml). The Petri dish (9 cm) containing medium (20 ml) and flower extract was inoculated with 0.5 ml spore suspension prepared from 5 day old culture and incubated for 5 days at 28±2°C under dark. Three replications were prepared for each treatment. For bioassay, groundnut seeds were coated (soaked for 30 min.) with the flower extract of different concentrations and inoculated with *A. flavus* and *A. parasiticus* fungi in SDA medium. The experiment was conducted in three replications for each concentration of flower extract of *E. splendens* and *L. nepetaeJolia*.

Growth of aflatoxin-producing fungi *A. flavus* (NCBT 101) and *A. parasiticus* (NCBT 128) were inhibited by treatment with flower dry powder of *E. splendens* and *L. nepetaeJolia* (Table 1). Total inhibition (100%) was noticed in *A. flavus* treated

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Fig. 1 — Inhibitory effect of crude flower extract of *Euphorbia splendens* (A1 and A2) and *Leonotis nepetactifolia* (A3 and A4). Bioassay with groundnut seeds coated with crude flower extract of *Euphorbia splendens* (B1 and B2) and *Leonotis nepetactifolia* (B3 and B4). (a) — Control without flower extract (b) — Control with bavistin (5 mg/ml) (c) — Flower extract (5 mg/ml) (d) — Flower extract (10 mg/ml) and (e) — Flower extract (15 mg/ml).
with 10 and 15 mg/ml concentration of L. nepetaefolia flowers. Growth of A. parasiticus was inhibited to 75% by L. nepetaefolia. A similar trend (75% inhibition) was seen in control (ii) with bavistin treatment. A maximum of 75% inhibition was seen in A. flavus treated with 15 mg/ml concentration of E. splendens flowers followed by 50% inhibition with 10 mg/ml. Control (ii) with bavistin showed 75% inhibition of growth (50%) was also noticed in A. parasiticus treated with E. splendens flower dry powder for 5 mg/ml, whereas control (ii) with bavistin showed 75% inhibition. Bioassay with groundnut seeds coated with 5, 10 and 15 mg/ml flower extracts of E. splendens and L. nepetaefolia confirmed the fungitoxic nature, especially at 10 and 15 mg/ml concentrations (Fig. 1).

Table 1—Effect of crude flower extracts of Euphorbia splendens and Leonotis nepetaefolia on aflatoxin-producing fungi Aspergillus flavus and Aspergillus parasiticus at 28°C ± 2°C

<table>
<thead>
<tr>
<th>Plant</th>
<th>Fungus</th>
<th>Control (i) (5 mg/ml)</th>
<th>Control (ii) (5 mg/ml)</th>
<th>Concentration mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euphorbia splendens</td>
<td>A. flavus</td>
<td>++++</td>
<td>+++</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>A. parasiticus</td>
<td>++++</td>
<td>+++</td>
<td>10</td>
</tr>
<tr>
<td>Leonotis nepetaefolia</td>
<td>A. flavus</td>
<td>++++</td>
<td>++</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>A. parasiticus</td>
<td>++++</td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>

Normal growth (+++); 25% growth inhibition (+++); 50% (++); 75% (+); and 100% (-).

Control (i)—Medium without flower extract; and control (ii)—Medium with bavistin fungicide.

Total inhibition of growth of A. flavus for L. nepetaefolia flower dry powder was possibly due to interference by the aromatic compounds present in the flower. Such interference may be at the biosynthetic levels. The level of inhibition differs between A. flavus and A. parasiticus. Earlier reports 7,8 have suggested that growth and aflatoxin production by A. flavus and A. parasiticus are independent phenomena. The isolation and characterization of the compounds present in L. nepetaefolia, capable of inhibiting aflatoxin producing fungus A. flavus, would be useful, since success in this area could provide a means for the control or elimination of aflatoxin-contamination of food stuffs 9. In conclusion, L. nepetaefolia inhibited significantly the growth of A. flavus and A. parasiticus as compared to E. splendens. If inhibitory factor(s) could be examined at biosynthetic level, L. nepetaefolia flower dry powder (or) extract might be useful in controlling aflatoxin contamination in food and feed.

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Reference