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Efficacy of *Euphorbia splendens* and *Leonotis nepetaefolia* 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. nepetaefolia* 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. nepetaefolia* 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 15mg/ml of *L. nepetaefolia* flower extracts.

Keywords: Aflatoxin, *Aspergillus flavus*, *Aspergillus parasiticus*, *Euphorbia splendens*, *Leonotis nepetaefolia*, 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[1,2]. Many plant extracts have been used to study their antifungal properties and effects on mycotoxin production, and anti-aflatoxin properties[3,4]. *Euphorbia splendens* (Euphorbiaceae) is a small perennial prickly much branched shrub with showy crimson flowers, and *Leonotis nepetaefolia* (Lamiaceae) is a tall herb with orange-scarlet flowers[5]. 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 nepetaefolia* 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 nepetaefolia* 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 100ml of sterile distilled water to prepare an aqueous extract[6]. 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. nepetaefolia*.

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. nepetaefolia* (Table 1). Total inhibition (100%) was noticed in *A. flavus* treated

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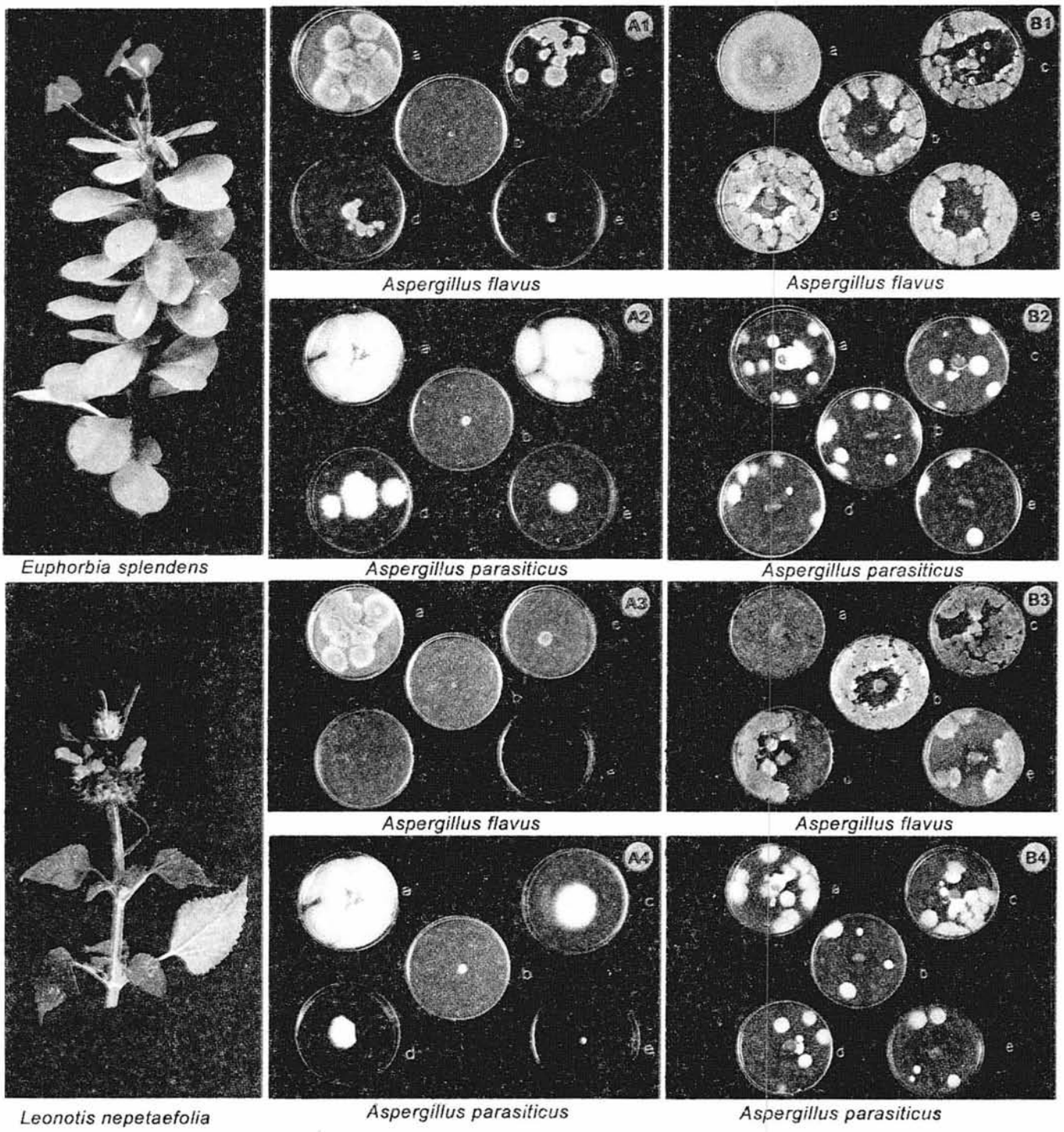

Fig. 1 — Inhibitory effect of crude flower extract of *Euphorbia splendens* (A1 and A2) and *Leonotis nepetaefolia* (A3 and A4). Bioassay with groundnut seeds coated with crude flower extract of *Euphorbia splendens* (B1 and B2) and *Leonotis nepetaefolia* (B3 and B4). (a) — Control without flower extract (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).

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

Plant	Fungus	Control (i) (5 mg/ml)	Control (ii) (5 mg/ml)	Concentration mg/ml 5	10	15
Euphorbia splendens	*A. flavus*	++++	+	+++	++	+
	A. parasiticus	++++	+	+++	++	+
Leonotis nepetaefolia	*A. flavus*	++++	+	++	–	–
	A. parasiticus	++++	+	++	++	+

Normal growth (++++); 25% growth inhibition (+++); 50% (++); 75% (+); and 100% (-).
Control (i) — Medium without flower extract: and control (ii) — Medium with bavistin fungicide

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 per cent inhibition with 10 mg/ml. Control (ii) with bavistin treated and 15 mg/ml of *E. splendens* flower dry powder also inhibited 75% of *A. flavus*. Reduction 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).

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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