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In vitro inhibition of growth and sporulation in *Aspergillus niger* by lidocaine – a local anaesthetic agent

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Lidocaine, a potent local anaesthetic (LA) caused a strong inhibition of mycelial growth, biomass production and sporulation in *Aspergillus niger* when cultured *in vitro*. The effects of the anaesthetic were concentration-dependent, although the dose response varied with the nature of the medium used. Fungal growth was more sensitive to lidocaine in liquid (peptone–glucose) than solid (peptone–glucose–agar) medium. The onset of sporulation was progressively delayed with increased concentration of anaesthetic, only in the former case. The fungistatic effect of lidocaine was apparent at 0.6% in liquid and at 0.9% in solid medium. The probable mode of LA-caused irreversible suppression of fungal growth has been discussed.

LOCAL anaesthetics (LAs) are drugs that cause local anaesthesia in animals by blocking nerve conduction[1–3]. Chemically, these are tertiary amines linked through an amide or ester linkage to the aromatic moeity. LAs act via blockade of voltage-dependent sodium channels by binding to intrapore receptor on the membranes, that hinders the generation of action potential responsible for nerve conduction[4]. The influence of LAs in a wide range of cellular responses even in the non-nerve tissues has been reported[5].

Plants utilize action potentials in regulating a variety of physiological responses such as ion movements[6,7], phloem unloading[8], opening and closing of stomata[9] and nastic and thigmotropic leaf movements[10]. An exposure to anaesthetics reversibly inhibited the motor mechanism operative in folding/unfolding of leaflet movements in *Mimosa pudica*[11]. In a recent study, an anaesthetic agent lidocaine has been shown to inhibit epiphyllous bud differentiation in *Kalanchoe pinnata*[12].

It was considered of interest to check the effect of LA, if any, on the colony growth and sporulation of *Aspergillus niger*, a fungal organism of high economic importance.

One-month-old *A. niger* (WB 326) was used for inoculum preparation. The slants with sporulating mycelia were flooded with 10 ml sterile water and gently rubbed with a sterile glass rod, then filtered through double layer of sterilized cheese cloth to remove agar bits and larger hyphal fragments. Spore concentration was determined by making replicative haemocytometer counts and density was adjusted to 1×10^6 ml^{-1}. The effect of lidocaine hydrochloride (2-diethylamino-*N*-[2,6-dimethylphenyl]-acetamide) procured from Sigma–Aldrich was studied on growth and sporulation of *A. niger* in solid as well as liquid media. Peptone–glucose–agar (PGA) medium was prepared by mixing 1% peptone, 2% glucose and 1% agar. Lidocaine was added to the medium prior to autoclaving to obtain final anaesthetic concentrations ranging from 0.1 to 1.0% w/v. Controls without lidocaine were also maintained. Petri dishes and media were autoclaved at 15 lb and 121°C for 15 min and point-inoculated centrally with *A. niger*. Peptone–glucose (PG) medium was prepared by mixing 1% peptone and 2% glucose. Lidocaine was added to the medium to obtain final anaesthetic concentrations ranging from 0.1 to 1.0% w/v, in addition to control. Flasks containing 50 ml of medium with varying concentrations of anaesthetic were autoclaved and subsequently inoculated with 0.05 ml of spore suspension.

All cultures were incubated for 6 d in dark at 25 ± 2°C. Treatments were replicated thrice and the experiment was repeated three times. Observations were recorded after 6 d for the extent of radial growth of the fungal colony as well as the central sporulation zone on solid medium and for fungal biomass production (fresh weight, dry weight) in the liquid medium. Daily observations were also recorded for the initiation of sporulation in all fungal cultures.

Data were subjected to one way analysis of variance (ANOVA) and multiple comparisons for difference in mean values of treatment and control were made by Turkey's test at $P \leq 0.05$, using Sigma Stat software[13] version 2.0.

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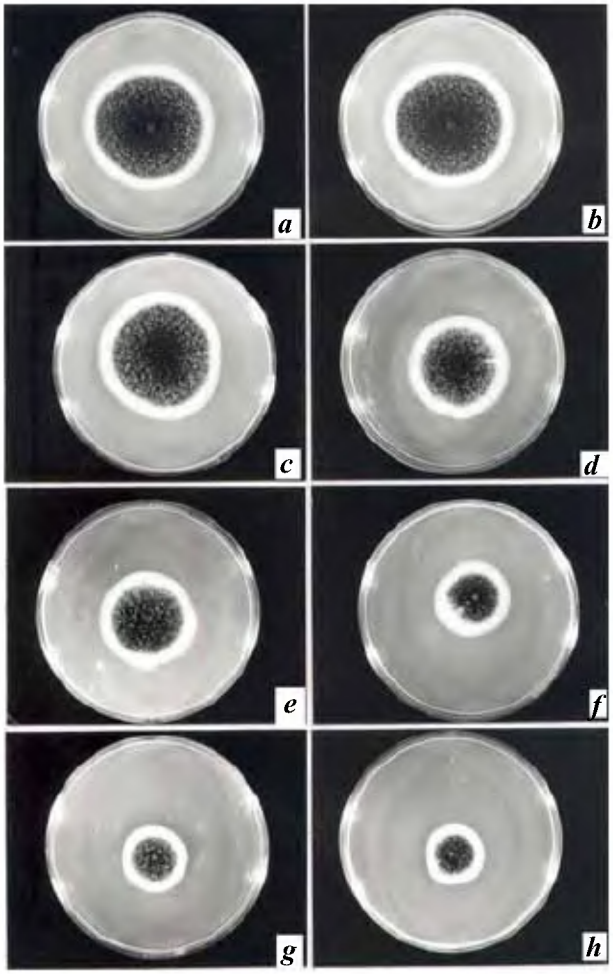

Figure 1. Radial colony growth and zone of sporulation of *Aspergillus niger* cultured for 6 days on solid (PGA) medium and supplemented with 0 (*a*), 0.1 (*b*), 0.2 (*c*), 0.3 (*d*), 0.4 (*e*), 0.5 (*f*), 0.6 (*g*) and 0.7 (*h*) % lidocaine.

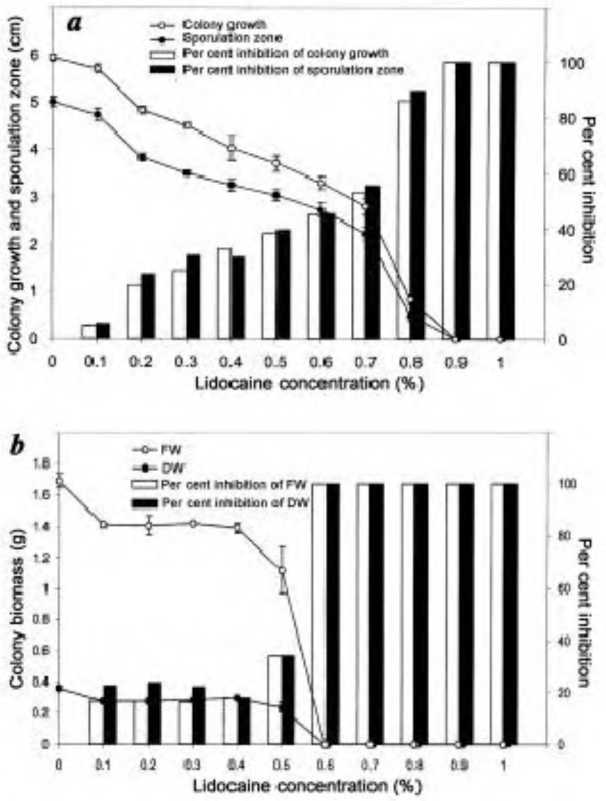

Figure 2. Effect of lidocaine on radial colony growth and zone of sporulation on solid medium (*a*), and on biomass production (FW and DW) in liquid medium (*b*), of *A. niger* cultured for 6 days without (control) and with different concentrations of lidocaine. Vertical columns depict per cent inhibition of these parameters caused by the anaesthetic at different dose levels. Bars represent standard error for triplicate cultures (significant at $P \leq 0.001$ or $P \leq 0.05$).

When point-inoculated on solid PGA medium, the resultant *A. niger* colony acquired a nearly geometric radial growth with a diameter of 5.98 ± 0.1 cm over a period of 6d. When the medium was supplemented with lidocaine, the colony diameter got reduced, the effect increasing with concentration (Figure 1). Thus, it was reduced to 0.83 ± 0.1 cm, i.e. by 86% at 0.8% lidocaine. At still higher concentrations no colony growth occurred (Figure 2 *a*).

The zone of sporulation extended up to 5 ± 0.1 cm of the colony diameter in case of the control. In lidocaine-supplemented media, the zone of sporulation also got reduced progressively with increasing concentration of anaesthetic, following a dose response similar to that seen for the radial growth of the colony (Figure 2 *a*).

The extent of lidocaine-caused inhibition of fungal growth and sporulation (calculated as reciprocals of values relative to untreated control taken as 100) pointed to steadily increasing effect with rise in anaesthetic concentration for both parameters investigated (Figure 2 *a*).

In liquid PG, *A. niger* colony grew from periphery towards the centre, eventually filling the surface of the medium in 6d. Addition of lidocaine restricted growth to the margins, the effect increasing with concentration up to 0.5%. At higher concentrations the fungal growth was completely inhibited.

After 6 d the fungal colony had acquired a fresh weight (FW) of 1.69 ± 0.04 g in unsupplemented PG medium; in lidocaine-supplemented medium, colony FW was reduced to 1.1 ± 0.2 g, i.e. by 33.9% at 0.5% lidocaine. However, a regular dose response decrease was not observed at increased concentrations (Figure 2 *b*). The differences in the mean values among the treatment groups were significant at $P \leq 0.001$.

The dry weight (DW) of control cultures after 6 d was 0.36 ± 0.03 g in liquid medium, whereas in lidocaine-supplemented medium (0.5% lidocaine), it was reduced to 0.24 ± 0.05 g, i.e. by 33.85%.

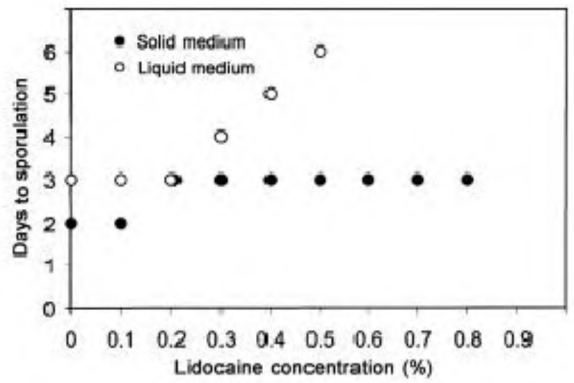

Figure 3. Effect of increasing lidocaine concentration on days to sporulation in *A. niger* cultured on either solid (PGA) or liquid (PG) medium.

The extent of lidocaine-caused inhibition of FW and DW (calculated as reciprocals of values relative to untreated control taken as 100) clearly exhibited the peculiar dose-dependent responses to the anaesthetic treatment (Figure 2 *b*).

It took 2 and 3 d for the initiation of fungal sporulation respectively, in the solid and liquid medium. However, the influence of lidocaine on sporulation of *A. niger* was different in solid and liquid growth conditions. In solid medium, it was not affected by 0.1% lidocaine and was delayed by a day at 0.2 to 1% level. In liquid medium, although sporulation was not affected at 0.1% anaesthetic, it got progressively delayed with rise in concentration from 0.2 to 0.5% (Figure 3).

The results obtained in this investigation show that treatment with lidocaine, a potent local anaesthetic agent brought about a strong inhibition of mycelial growth, biomass production and sporulation in *A. niger*. The inhibitory effect increased with increasing concentration of anaesthetic and was more pronounced in liquid than in solid medium. Action of lidocaine has a fungistatic effect, delaying the growth of fungal colony as well as initiation and extent of sporulation. In fact, 0.6% of the anaesthetic had a fungicidal effect in the liquid medium. All observed effects are long-term modulation of fungal growth and sporulation that would obviously involve an altered gene expression, induced by a local anaesthetic agent. These responses are unlike the short-term and reversible blockade of the nerve conduction seen in animals[3] and the motor mechanism implicated in the nastic movements of *M. pudica*[10,11], in either case operating through membrane potentials. In fact, these results are more in line with the LA-caused inhibition of various attributes of epiphyllous bud differentiation observed in *K. pinnata*[12]. Nevertheless, the probable involvement of membranes as an initial component of its mechanism for action in the present case needs further investigation, since ion channels have been detected in *Aspergillus*[14] and other fungal hyphae[15,16] with essential role in fungal growth and a possible involvement in Ca+ signalling[17,18], osmoregulation, organic acid efflux and pH homeostasis[19].

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