Yearlings of Indian major carps resist infection against the epizootic ulcerative syndrome pathogen, *Aphanomyces invadans*

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One-year-plus age groups (yearlings) of Indian major carps (IMC) were artificially infected with the epizootic ulcerative syndrome (EUS) pathogen, *Aphanomyces invadans* and detailed sequential inflammatory response against the pathogen was delineated. For comparison, the corresponding age groups of EUS-resistant (common carp, CC) and susceptible (snakehead) fish species were used in the artificial infection experiments. In all the fish species the injected spores were able to germinate, but in susceptible fish species the germinated hyphae were able to proliferate massively and cause extensive necrotic pathology leading to severe gross lesions. On the other hand, in IMC and CC, the germinated hyphae were prevented from significant proliferation and spreading to the neighbouring tissues and the lesion area was healed over the experimental period of 15 days and no gross visible lesion was observed. Higher number of inflammatory cells and more efficient epithelioid cell layer formation are believed to play an important role in the defence mechanism of yearlings of IMC and CC against *A. invadans* infection.

Keywords: *Aphanomyces invadans*, epizootic ulcerative syndrome, Indian major carps, yearlings.

EPIZOOTIC ulcerative syndrome (EUS) is one of the most destructive diseases of freshwater and brackish water-farmed and wild fish in the Asia-Pacific region. It has caused major fish loss over three decades[1]. The etiological agent of the disease is an oomycete fungus, *Aphanomyces invadans*[2,3]. More than 100 fish species have been reported to be affected by the disease and some commercially important species like common carp (CC), tilapias and milk fish are considered to be resistant[4]. However, the impact of the disease on Indian major carps (IMC; the major cultured fish species of the Indian subcontinent) has not been assessed properly. During EUS outbreaks in several southern[5–7], and north and northeastern states[8,9] of India, IMC present in many water bodies has been observed to be unaffected. High temperature in South India has been suggested as one possible factor responsible for increased resistance of IMC to *A. invadans* infection[10]. Temperature alone may not answer some of the observations made in the northeastern states of India, which are ideal for EUS outbreaks. The possibility that age or size might be important in the susceptibility of the IMC to EUS was suggested by Lilley *et al.*[4], and Chinabut and Roberts[11]. However, there is no published evidence to support these suggestions. On the other hand, in some other susceptible species like snakehead, size or age appears to have no relation to the degree of susceptibility[4]. Therefore, in the present study, yearlings of three species of IMC (catla, *Catla catla*; rohu, *Labeo rohita*, and mrigal, *Cirrhinus mrigala*) along with corresponding age groups of EUS-resistant (CC, *Cyprinus carpio*) and susceptible (snakehead, *Channa striatus*) fish species were artificially infected with zoospores of *A. invadans*. The objectives were: (i) to know whether yearlings of IMC are resistant to *A. invadans* infection and (ii) to compare their inflammatory response with EUS-resistant (CC) and susceptible (snakehead) fish species to find out the differences, if any.

Thirty yearlings of catla, rohu, mrigal and CC each (averaging 26.9 ± 2.2, 35.2 ± 2.9, 20.1 ± 1.5 and 21.2 ± 2.1 cm respectively, in body length) and 18 snakeheads (averaging 20.9 ± 2.4 cm in body length) were used for artificial infection test. The experimental carps (catla, rohu, mrigal and CC) were collected from Bhadra Fish Seed Farm, Department of Fisheries, Karnataka, at fry stage in the first week of July 1999. Snakeheads (*Channa* spp.) were also collected at fry stage, from the nearby paddy fields in Mangalore, during the same time period. All the experimental fishes were reared in the fish farm at the College of Fisheries, Mangalore, for a period of 12 months. During the rearing period of one year, fishes were not exposed to *A. invadans* and also no incidence of EUS was reported in the region. Experimental infection studies were carried out in July 2000. Water temperature of the experimental tanks ranged from 26 to 29°C as measured twice daily in the morning and evening.

Suspension of motile secondary zoospores of *A. invadans* (strain B99C kindly provided by J. H. Lilley) was prepared as described by Lilley *et al.*[4]. All the fish species were divided into experimental and control groups having equal number of fishes. Each experimental fish was injected intramuscularly with 0.2 ml of spore suspension (6×10^4 spores per ml) of *A. invadans* (strain B99C), as described by Chinabut *et al.*[12]. The control fish group received 0.2 ml autoclaved pond water at the same time as the test fish. After injection, each fish species of experimental and control groups was kept in separate cement cisterns of 5 × 5 sq. m size containing water (about 0.9 m depth) for 15 days. Three fishes each from the experimental and control groups were sampled at 3, 6, 9, 12 and 15 days post infection (dpi). In the case of snakehead, due to low number of fish available, sampling was not

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made along with IMC, however, only the moribund fish were sampled. After gross observation of the sampled fish, blocks of muscle and skin were excised from the area of injection and fixed in 10% neutral buffered formalin and all the histopathological analysis was carried out as described by Chinabut and Roberts[11].

In none of the IMC and CC were any gross visible lesions observed during the 15-day period. On the other hand, in snakehead (*Channa* spp.) by 9 dpi, all the fishes had typical gross lesions characterized by severe swollen haemorrhagic ulcerative areas at the site injection. Histopathological studies indicated that in the case of IMC, on 3 dpi, extensive inflammatory cellular infiltrate dominated by macrophages had migrated into the lesion area and most of the hyphae were encapsulated by inflammatory foci of microphages (Figure 1 *a*). By 6 dpi, active myophagia was observed in the degenerated muscle fibres and all the hyphae in the lesion area were encapsulated by well-developed epithelioid cell granulomata (Figure 1 *b*). Hyphae within the granulomata appeared crumbled (Figure 1 *b*) and around few of the granulomata extensive inflammatory cellular infiltrate were observed (Figure 1 *c*). After 9 dpi, the degenerated muscle fibres were cleared by macrophages and the lesion area was replaced with many regenerated muscle fibres (Figure 2 *a–c*). By 12 and 15 dpi, the mycotic lesion area was well organized and replaced with well-developed regenerated muscle fibres, and biological activities of the hyphae within the granulomata appeared completely suppressed (Figure 3 *a–c*).

In the case of CC, distinctive floccular degeneration of muscle fibres as observed in the IMC was not found. By 6 and 9 dpi, the lesion area appeared to have healed with numerous regenerated muscle fibres (Figure 4 *a*) and the lesion area had completely healed by 12 dpi. In the case of snakehead, at 9, 12 and 15 dpi, the histopathological features were typical of a disease condition. There was no evidence of healing or regeneration of muscle fibres. There was severe myonecrosis of large areas of myotome (Figure 4 *b*) and massive proliferation of hyphae. Unlike in IMC, the hyphae within the granulomata did not appear crumbled (Figure 4 *c*).

From these findings, it can be concluded that yearlings of IMC and CC could resist artificial infection with *A. invadans*. Since a similar concentration of spores from the same batch was injected to all the fish species, it appears that in IMC and CC, some mechanism(s) would have prevented most of the spores from germinating. Kurata *et al.*[13] observed that CC serum has fungicidal activities, which support such an argument. Further, from the present study it is clear that in susceptible species (snakehead), the germinated hyphae were able to proliferate massively and cause extensive necrotic pathology leading to severe gross lesions. On the other hand, in IMC and CC, the germinated hyphae were prevented from significant proliferation and spreading to the neighbouring tissues. As a result, the lesion area was confined and healed by a well-organized inflammatory response. Papadimitriou and Spector[14] suggested that macrophages develop epithelioid characteristics when the influx of macrophages into an inflammatory site exceeds the number required to phagocytose an indigestible irritant. Therefore, it appears that the macrophages cannot phagocytose the large fungal hyphae,

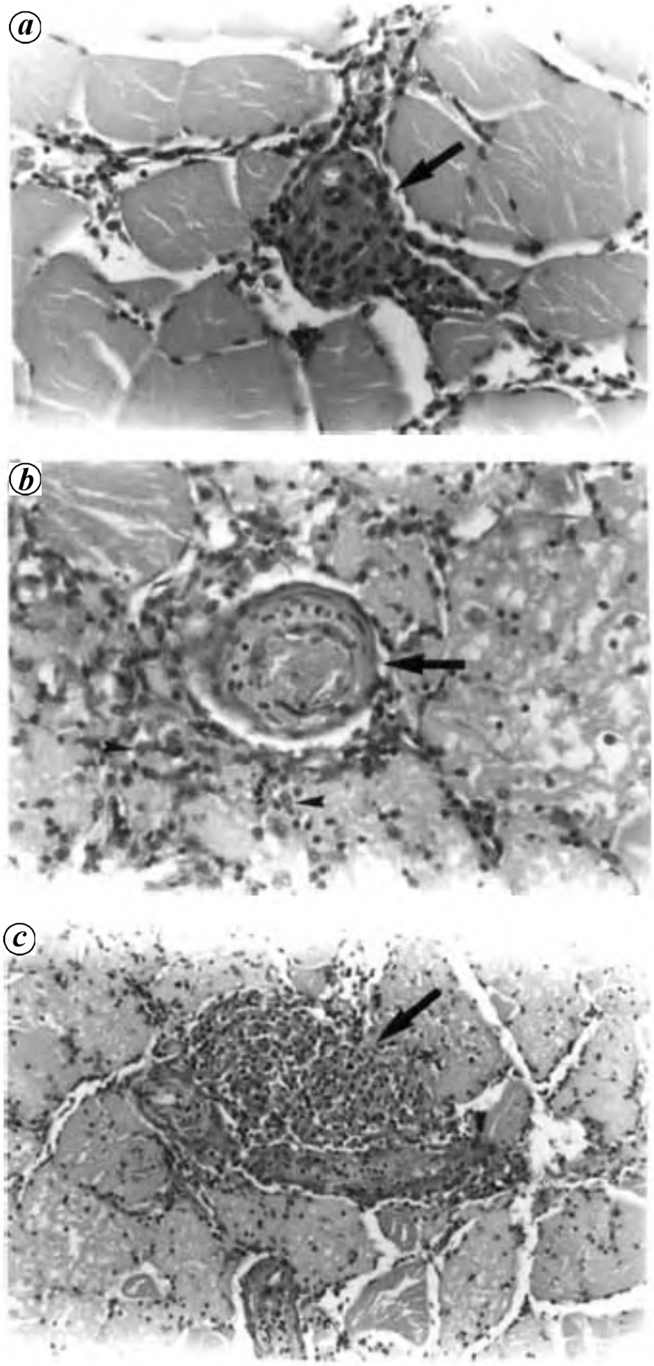

Figure 1. ***a***, Inflammatory focus of macrophages around the hyphae (arrow) at 3 days post injection (dpi) in mrigal (H&E, × 400). ***b***, Well-developed epithelioid cell granuloma (arrow) and myophagia in the degenerated muscle fibres (arrowheads) at 6 dpi in catla (H&E, × 400). ***c***, Extensive inflammatory cellular infiltrate (arrow) around epithelioid cell granuloma in catla at 6 dpi (H&E, × 200).

but modify themselves to epithelioid cells as a strategy to suppress their biological activity. Comparison of morphological features of epithelioid cell granulomata between different species indicated that in the case of IMC, the epithelioid cell layers around the hyphae were thick. This may be due to infiltration of a large number of macrophages into the lesion area. Interestingly, even though extensive inflammatory cells were observed in the lesion area of snakehead, tightly bound epithelioid cell layers as observed in the yearlings of IMC were not seen. Also, comparison of the status of hyphal matter within the epithelioid cell granulomata indicated that in IMC, the hyphal matter appeared completely crumbled. No such crumbling was observed in snakehead. Spector and

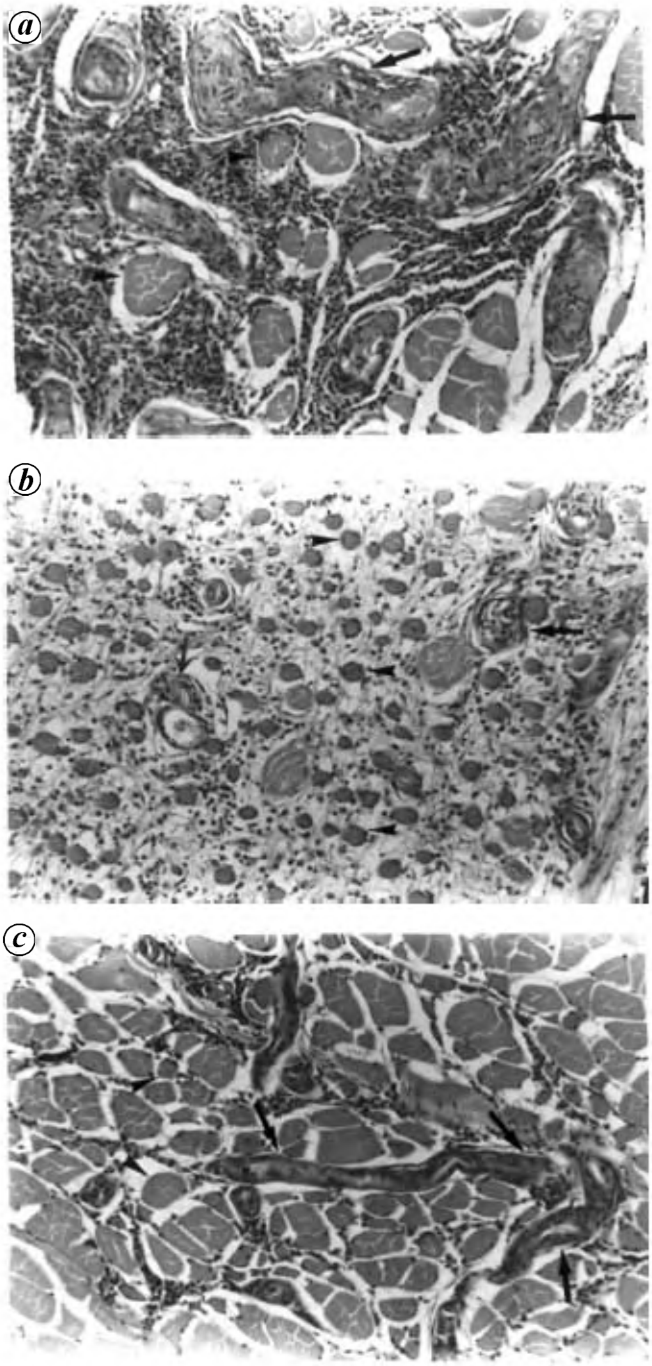

Figure 2. ***a***, Lesion area showing extensive inflammatory cellular infiltrate (arrows) and many regenerated muscle fibres (arrowheads) in catla at 9 dpi (H&E, × 200). ***b***, Regenerated muscle fibres (arrowheads) and epithelioid cell granulomata (arrows) in the lesion area of rohu at 9 dpi (H&E, × 200). ***c***, Lesion area of mrigal at 9 dpi showing regenerated muscle fibres (arrowheads) and fungal hyphae (arrows) (H&E, × 200).

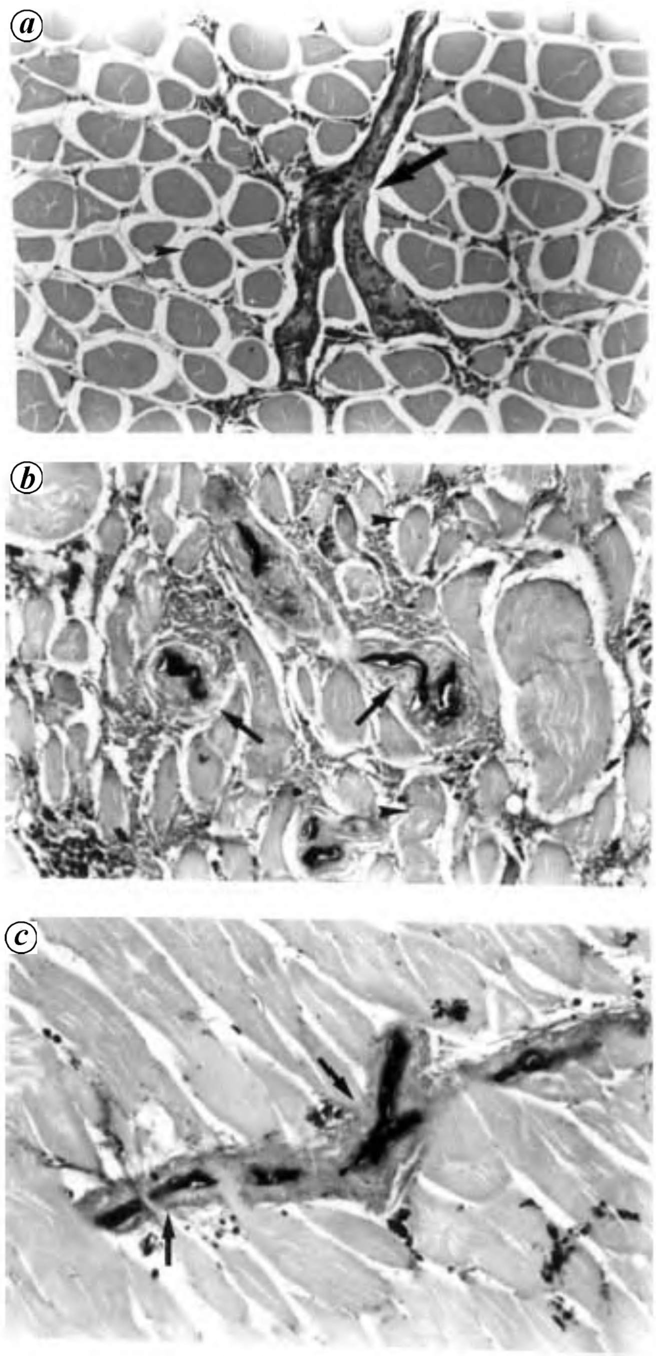

Figure 3. ***a***, Well-organized lesion area showing fungal hyphae (arrow) and adjacent normal muscle fibres (arrowheads) in mrigal at 12 dpi (H&E, × 200). ***b***, Crumbled hyphal matter within the granulomata (arrows) and well-developed regenerated muscle fibres (arrowheads) at 12 dpi in rohu (Grocott – H&E, × 400). ***c***, Longitudinal section of a granuloma (arrows) showing crumbled hyphal matter inside and adjacent normal muscle fibres in mrigal at 15 dpi (Grocott – H&E, × 200).

Mariano[15] reported that epithelioid cells are poorly phagocytic, but have an increased capacity for intracellular digestion and enzyme secretion. Therefore, it appears that in IMC the mechanism(s) might be different from that of susceptible species.

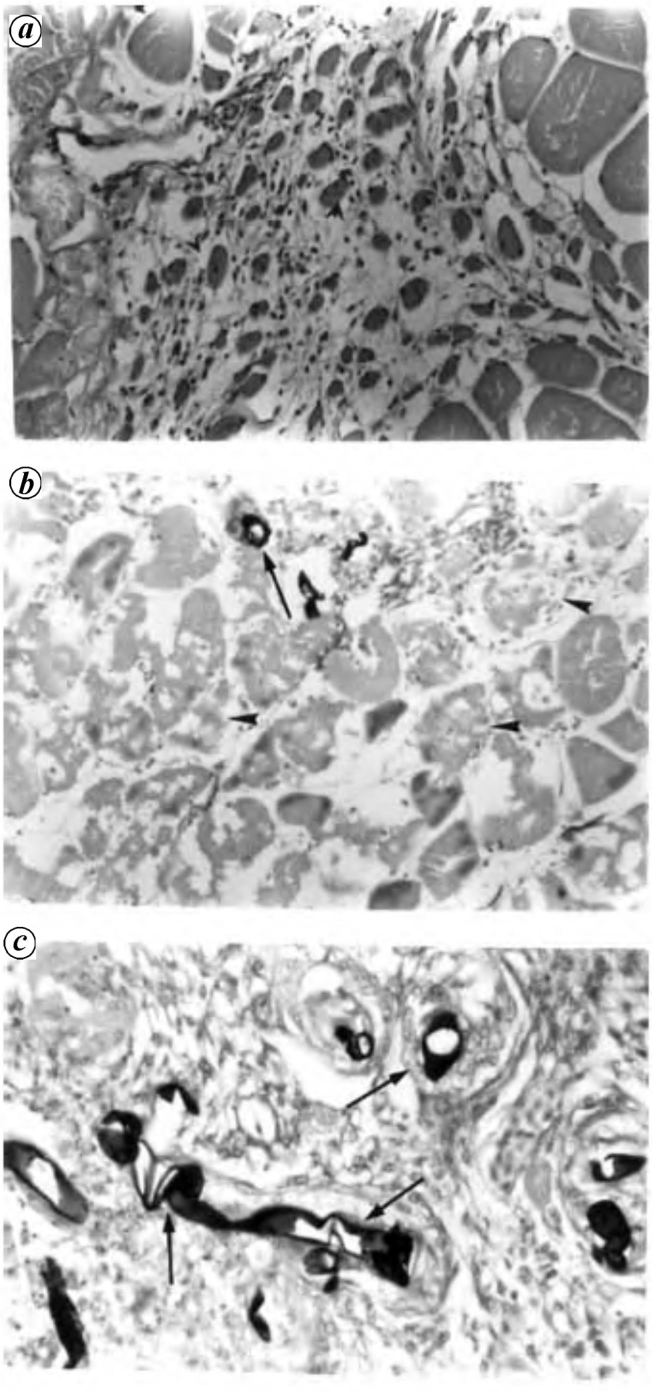

Figure 4. ***a***, Lesion area appears healed with regenerated muscle fibres in CC at 6 dpi (H&E, × 200). ***b***, Degenerated muscle fibres (arrowheads) in the lesion area of snakehead at 15 dpi (Grocott – H&E, × 200). ***c***, Encapsulation of fungal hyphae by several layers of epithelioid cells (arrows) at 15 dpi in snakehead. Compare the nature of hyphal matter with that of the hyphal matter within the granulomata of IMC (Grocott – H&E, × 400).

In IMC, around many longitudinal sections of the hyphae, the typical myonecrosis of the adjacent muscle fibres (assumed to be associated with the release of proteolysins by the fungus) was absent (Figure 3 *a–c*). However, in snakehead, around the hyphae there was extensive myonecrosis (Figure 4 *b*, *c*). The presence of viable mycelial tips in the tissue may be the reason for extensive myonecrosis. Absence of myonecrosis around fungal hyphae in IMC might suggest that the hyphae were no longer viable. It appears that the epithelioid cells in yearlings of IMC might play an important role in cellular defence mechanisms to eliminate the inoculated fungus. In IMC, around some of the granulomata, extensive accumulation of inflammatory cells was observed. So it was considered that some hyphae might have escaped from the defence mechanism of epithelioid cell granulomata and tried to extend to the neighbouring tissue, and extensive infiltration of inflammatory cells would have suppressed their activity. Therefore, from the present study, it was concluded that higher number of inflammatory cells and more efficient epithelioid cell layer formation play an important role in the defence mechanism of yearlings of IMC against *A. invadans* infection.

In extensive observational studies during EUS outbreaks in Karnataka (South India), it was found that IMC present in many water bodies was not affected[6,7]. Similarly, in Tamil Nadu (South India), IMC present in culture ponds was also reported to be unaffected during EUS outbreaks[5]. Chinabut and Roberts[11] have reported that there is an anomaly in susceptibility of IMC with regard to geography (i.e. IMC appears resistant in South India, but not in North India). Roberts *et al.*[10] suggested that the temperature in South India was high enough for the IMC to resist infection.

Surprisingly, during EUS outbreaks in several north and northeastern states, IMC was found refractory or mildly affected (whereas other species like *Puntius*, *Channa*, *Mastacembalus*, etc. were found severely affected)[8,9]. A review of the literature clearly indicated that EUS outbreaks (in freshwater fishes) generally occur during winter months (October to January) coinciding with a fall in water temperature[4]. In all the north and northeastern states of India, during winter months the temperature normally goes below 20°C, which is ideal for EUS outbreaks.

Therefore, if we go by the temperature hypothesis alone, in the north and northeastern states of India during winter months the temperature is ideal for EUS outbreaks, and the IMC should have been affected! Further, in artificial-infection studies with lower age groups of IMC[16] at Mangalore, it has been reported that the fingerlings and advanced fingerlings of IMC suffer heavy mortality. Hence, it was hypothesized by the authors that the unaffected or refractory IMC observed during EUS outbreaks in the affected water bodies in different parts of the country, could be of higher age groups and age/size might play an important role in the susceptibility of the IMC to EUS. The present experimental findings clearly indicate that the higher age groups (yearlings) of IMC are able to resist artificial infection with *A. invadans* (whereas the same age groups of snakehead were susceptible).

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Weed floristic composition in palm gardens in Plains of Eastern Himalayan region of West Bengal

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Weeds are unwanted plants in the crop land that compete for nutrients, water and space. Proper knowledge about weed flora is important for their management. Weather conditions in sub-Himalayan West Bengal favour weed growth. Therefore, a study was conducted to find out the weed floristic composition of different palm gardens in this region. The results showed that dicots were predominant in the palm gardens. Maximum number of weeds was found in the oil palm gardens and the least in the fruiting arecanut gardens. A total of 20 angiosperm families were found in the study area. Among them, 17 belonged to dicots and three to monocots. A total of five pteridophytes were found. Members of Poaceae, Asteraceae, Oxalidaceae and Urticaceae were found in all the plots studied. Three species, viz. *Ageratum conyzoides*, *Oxalis corniculata* and *Vandelia* were found to be more widely distributed in all four palms as well as in fallow land, showing Shannon's index value >0.75. Prevalence of some weeds in all the study areas revealed that they can grow under any conditions.

Keywords: Control, plains, Eastern Himalayas, palm gardens, weeds.

WEEDS compete with other crops for water, nutrients and space. Weeds also act as alternate hosts for pests and diseases. The abundance or distribution of weed species in a cropped field varies due to the nature of the crop, cultural practices and cropping pattern/system, soil type, moisture availability, location and season. Knowledge of weed flora enables one to use the required herbicide and formulate other suitable management strategies. It is also useful in exploiting abundant weeds as a cover crop or pasture and for other economic uses. Extensive literature is available on weed flora dynamics in field crops[1–3], as well as on weed flora in palms grown as plantations[4,5], date palm[6,7], arecanut[8] and peach palm[9,10]. Control of weeds by hand-weeding or application of herbicides is essential for better crop yield. However, one should have a clear idea about the existence of different weed flora under the shade of different plantation crops like arecanut, coconut and oil palm. According to Derksen *et al.*[1], the study of weed dynamics is essential to formulate a management strategy for

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