rhodozyma along with β-carotene, zeaxanthin, lutein, salmoxanthin, etc. All of which are supplemented well with L-ascorbic acid, 2-glucoside, potato starch, soyabean meal, krill powder and other base substances. Fish given this feed appears to accumulate more of the pigment in the tissue and thus appears red which naturally draws better market. Astaxanthin-supplemented diet has been found to shorten the moulting cycle of the prawn *Panaeus japonicus*[5]. Carotenoids avoid lipid peroxidation which increases the shelf life of the fish. β-carotene is also employed as the source of vitamin A for those fishes which can split the carotene molecule. Care should be taken to avoid oxidation and degradation of the carotenoids and is done by incorporating glutathione in the feed.

When the importance of the micro-organisms as feed became noticeable, lots of trials were also done to get best growth of the micro-organisms. in the recent years, *Phaffia rhodozyma*[3,4] has been studied and mutagenized with the help of the mutagens such as ethylmethylsulphonate, NTG, and by UV to produce high quantity of the astaxanthin. Cultural conditions are also modified to optimize growth. It is shake cultured in the medium containing yeast extract, polypeptone, sucrose at 20°C for three days and at 25°C for five days. Chlorella[6] is grown in the transparent bioreactor under aeration. This enables fast and high density growth of the algae. Fish normally prefer feed in granulated form which is obtained by treating it with alkali. Alkali treatment to the feed also gives the stability which does not allow it to get dispersed into the water, thus avoiding pollution. Algae and yeasts were subjected to the alkali treatment immediately after achieving suitable growth with sodium hydroxide to achieve pH of 12.5 (ref. 7). This treatment replaces the use of alkali metal salt. *Rhodobacter capsulatus* is employed as the secondary feed for Japanese flounder larvae and juveniles. It was found to act as an antioxidant which decreases the formation of the lipid peroxide in the juveniles[8]. A major part of the normally employed fish feed having squid viscera, plant material such as corn gluten meal is fermented with yeast and molds to get better feed. The disease resistance of fish can be strengthened by using better quality fish feed and can minimize use of antibiotics like chloramphenicol[9] in the fish feed. Use of micro-organisms as fish feed is found safe for human consumption. Thus micro-organisms employed as the fish feed agent present a bright prospects for fish industry and to human being too.

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SCIENTIFIC CORRESPONDENCE

Association of *Frerea indica* Dalz., an endangered plant species with *Euphorbia neriifolia* L. and its importance in habitat conservation

Frerea indica, often described as an illusive plant[1], is a monotypic genus in the family Asclepiadaceae. The species is believed to be one of the rarest plant species[2–11] and is listed as one of the 12 most endangered plants on earth by the International Union for Conservation of Nature and Natural Resources. The distribution of the species is restricted only to Maharashtra state in India. The distribution range is limited to six localities of three districts, namely Pune, Satara and Raigad. Except Mahabaleshwar, the presence of the species is confirmed in the other four localities. The species is basically a high-altitude plant (> 1000 m), with a characteristic distribution in nature. A typical habitat of *F. indica* consists of steep slopes (> 60°), preferably the crest region with a thin layer of soil between lateritic rocks (Figure 1 *a*, *b*). Except fire, the habitat is in general, inaccessible, and is free from human disturbance[12].

In all the localities studied, *F. indica* was found associated with *Euphorbia neriifolia* (Euphorbiaceae), growing either under its bushes or somewhere closer. *F. indica* plants resemble young *E. neriifolia* plants and it is difficult to distinguish them. In fact, this has been the experience of earlier workers as well[13,14]. Other plant species found in the habitat include *Notonia grandiflora* DC., *Carvia callosa* Bremek., *Lantana camara* L., *Ensete superbum* (Roxb) Cheesman, *Chlorophytum* spp. and grasses. During our attempts in conservation of endangered species, we propagated *F. indica* in nurseries at NGCPR, Shindewadi. We found all the plants infested and severely damaged by the caterpillars of the milk weed butterfly, *Danus chrysippus*. Milk weed butterflies normally feed on species of Asclepiadaceae, including *Calotropis*[15]. However, we had not encountered these caterpillars on *F. indica* in its natural habits during our field studies. This prompted us to hypothesize, whether the presence of *E. neriifolia* plants in the vicinity of *F. indica* in the natural habitats in some way protects the latter species.

To test this hypothesis we conducted two related experiments. In the first experiment, we quantified the association relationship between *F. indica* with *E. neriifolia* in its natural habitat. In the second experiment, we tried to find out whether *E. neriifolia* exhibits any repellent properties to caterpillars of plain tiger.

For the association studies we collected

Figure 1. A typical habitat of *F. indica* locations. *a*, Junnar (Shivaneri) and *b*, Vazir Garh.

Table 1. Distribution, frequency, density, abundance and association index of *Frerea indica* and *Euphorbia neriifolia*

Locality	Total no. of *F. indica* plants		*F. indica*	*E. neriifolia*	Association index	Remarks
Junnar (Shivaneri)	65	Frequency (%)	100	100	8.14	Part of the habitat is inaccessible
		Density	3.6	4.6		
		Abundancy	3.6	4.6		
Purandar (Vazir Garh)	100	Frequency (%)	100	100	4.00	Part of the habitat is inaccessible
		Density	2.0	6.3	–	
		Abundance	2.0	6.3	–	
Sajjan Gad	39	Frequency (%)	100	–	–	Habitat is disturbed by fire. *E. neriifolia* is absent
		Density	2.3	–	–	
		Abundance	2.3	–	–	
Varandha Ghat (Shivtharghal)	1	–	–	–	–	Habitat is disturbed by fire. Only one plant was located, found in *E. neriifolia* habitat

ecological data from all reported sites in order to study frequency distribution, density analysis, abundance and association relationship of *F. indica* with *E. neriifolia* in its natural habitat. The results of the study are presented in Table 1.

For the second experiment to test *E. neriifolia*'s role in protecting *F. indica* from predator caterpillars of plain tiger, we conducted a simple experiment in which different combinations of *F. indica* and *E. neriifolia* leaves were fed to caterpillars. Observations are presented in Table 2. The experiment was conducted at NGCPR's laboratory at Shindewadi.

Quadrat analysis for association index (a simple χ^2 test from 2×2 contingency table) was made on the basis of the method suggested by Ludwig and Reynolds[16]. As the total number of plants of *F. indica* in its natural habitat is about 200, the quadrats of size 5×5 m were drawn in the localities only where *F. indica* was present. The number of quadrats varied from 5 to 6 depending upon the size of the population. *E. neriifolia* is the most commonly found thorny shrub on degraded hill slopes in high rainfall regions of Western Ghats of Maharashtra. The species is also widespread and commonly planted along farm boundaries. The density and abundance of *F. indica* are less, whereas *E. neriifolia* is abundant

Table 2. Observation on feeding habit of plain tiger caterpillar on *Frerea indica* and *Euphorbia neriifolia* leaves

Food material	No. of caterpillars	Observations
F. indica	5	Consumed all leaves within few hours
F. indica and *E. neriifolia*	5	Consumed only *F. indica*
E. neriifolia	5	Did not feed on leaves and settled near the rim, preferred starvation death

at all the sites. The association between *F. indica* and *E. neriifolia* can be termed as a complete association. The association relationship of *F. indica* is very strong since this species is endangered and association index is calculated for those *F. indica* plants that are growing under *E. neriifolia* bushes. Two sites, i.e. the Sajjan Gad and Varandha Ghat are found to be disturbed by fire. Hence association index is prepared for only two sites, viz. Junnar and Purandar (Table 1).

In the next experiment, we collected 15 healthy caterpillars of *D. chrysippus* (about 1.5 cm length) feeding on *F. indica* and allowed them in batches of five each in three petri dishes. In the first petri dish, we placed only *E. neriifolia* leaves. In the second, a combination of *E. neriifolia* and *F. indica* leaves was released. In the third, we released only *F. indica* leaves. The caterpillars of the first petri dish after initial nibbling preferred to stay at the rim of the petri dish, but never fed on *E. neriifolia* leaves. They preferred starvation death. In the second petri dish, the caterpillars fed only on *F. indica* leaves. In the third, the caterpillars fed on the *F. indica* leaves, completed their development on continuous supply of leaves from the same species, and pupated. The duration of the experiment was about 17–22 days.

The leaf and stem extracts of *E. neriifolia* are known to contain triterpene-glut5(10)-en-l-one in addition to friedelan-3 and 3B-ol and taraxerol, which showed significant local anesthetic activity in guinea pigs and withdrawal reflex in frogs[17]. Our preliminary experiment has shown that *E. neriifolia* has some repellence to *D. chrysippus* larvae. And, we presume that this factor resembling young *E. neriifolia* combined with thorny habit protects *F. indica* plants from plain tiger in its natural habitat. Growing in the *E. neriifolia* habitat has tremendous advantage for *F. indica* as far as its survival is concerned. We suggest here that while attempting reintroduction of *F. indica* or conserving its habitat, it is important to keep in mind the value of *E. neriifolia* association for this endangered species.

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