Table 2. Range of abundance of phyto and zooplankton densities in the early and late transition periods

Phytoplankton species	Early transition (cells ml $^{-1}$)	Late transition (cells ml^{-1})
Pennales		
Thallassionema nitzschioides	6–142	0
Nitzschia closterium	11–405	0–88
Thalassiothrix longissima	0–60	0–30
Asterionella glacialis	0–1376	0–18
Navicula longa	0–243	0–20
Nitzschia seriata	0–263	0–150
Amphora lineolata	0–25	0
Centrales		
Skeletonema costatum	0	0–159
Thalassiosira subtilis	0–3	0–48
Coscinodiscus excentricus	0–20	0–12
Chaetoceros didymus	0–50	0–94
Chaetoceros pelagicus	0–7	0–134
Zooplankton groups	**Early transition (animals m^{-3})**	**Late transition (animals m^{-3})**
Hydromedusae	0–176	0
Holoplankton		
Calanoids	1280–5760	388–3200
Non-calanoids	112–3096	80–576
Meroplankton	56–1376	32–248

in the plankton composition as evidenced by this study can well be treated as an indication of the arrival of these water bodies on this coast.

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Received 3 August 1999; revised accepted 16 October 1999

Control of coconut black-headed caterpillar (*Opisina arenosella* Walker) by systemic application of 'Soluneem' – A new water-soluble neem insecticide formulation

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The coconut black-headed caterpillar (bhc) *Opisina arenosella* is one of the major pests of coconut palms causing considerable damage to coconut industry. As effective control has so far been elusive, the discovery of a successful method of control of this pest by systemic application of 'Soluneem', the first water soluble, non-toxic neem pesticide is reported. A single dose of systemic administration of the formulation containing 3000 ppm of azadirachtin A in aqueous solution at the base of the trunk translocated the biopesticide to the crown within 24 h. A highly significant reduction in the larval population, moult inhibition, reduction in adult emergence and malformation in the emerged adults was recorded in Soluneem-treated trees. The protection lasted for more than 120 days with no phytotoxic symptoms to the treated palms and Soluneem was safe for natural enemies.

COCONUT black-headed caterpillar (bhc) *Opisina arenosella* Walker (Lepidoptera: Oecophoridae) is one of the major pests of coconut causing considerable dam-

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age to coconut industry and according to a recent survey, about 1.6 million palms are affected in Karnataka alone. Available control measures include mainly topical sprays and systemic application (root administration) of synthetic pesticides[1]. The latter method has been shown to leave persistent pesticide residues[2]. Consumption of tender coconuts from trees applied with synthetic pesticides may pose health hazards. In spite of several concerted efforts made for the past two decades to control the pest by synthetic insecticide application, the anticipated results have never been achieved. In this communication, we report the effectiveness and superiority of Soluneem over other neem formulations for the management of this notorious pest by systemic application.

Neem seed kernel extract is known to have potent insecticidal properties[3]. The insolubility of the active constituent azadirachtin in water and its poor stability limited the use of neem as a safe and effective insecticide for systemic application. Recently, we have developed the first water soluble eco-friendly neem pesticide formulation in a powder form (Soluneem) containing azadirachtin and other limonoids, which are otherwise very sparingly soluble in aqueous solutions[4]. Soluneem was found to be very safe (LD50 > 5 g/kg body wt), highly stable and effective in the control of a number of pests of economically important crops such as paddy, vegetables and ornamentals by foliar spray.

Soluneem along with three commercially available neem-based formulations, viz. Neemark, Nimbecidine and Neemazal F were selected to study their systemic absorption by coconut palms and effectiveness in the control of bhc infestation in a private farm at Chaluvanahally, Arsikere taluk, Karnataka, a heavily infested site with bhc since December 1995. The 'syringe method'[5] was used to systemically deliver the insecticidal formulations. Randomly selected coconut trees were used to study the effect of absorption rate of the formulations and their phytotoxic effects. For each of the formulations, four trees were selected for administration of the test chemicals. Fifteen hundred ppm of all the four formulations suspended/dissolved in 10 ml of mineral water were uniformly administered using 20 ml syringes between 8 and 9 am during July 1999. Observations were made on the absorption rate at 6 h intervals over a 24 h period. The treated trees were monitored for 14 weeks to record phytotoxic effects including any abnormal development like button shedding, yellowing, drooping or drying of leaves.

Two Soluneem-treated trees were sacrificed after a week of treatment to check for phytotoxicity at the administration site, adjoining tissues, and in the xylem bundles. To monitor the movement of the insecticide, a mixture of methylene blue (3 g) and Soluneem (1500 ppm) dissolved in 20 ml of mineral water was also administered to two more palms and after 24 h they were cut open to trace the movement of the dye. Appearance of blue colour in the xylem vessels in the trunk at different heights was used as an indicator for the upward translocation of Soluneem[5]. The absorption of Nimbecidine, Neemark and Neemazal F in 24 h duration was 1, 0.5 and 2 ml, respectively. The unabsorbed emulsion of these formulations was clearly visible in the syringe as two layers. Since the absorption was very poor, no further observations were made with these commercial formulations. The absorption of Soluneem (10 ml) on the other hand, was complete within 18 h. Two of the Soluneem-treated trees when cut open appeared normal and histological examination did not show any symptoms of phytotoxicity. Methylene blue dye mixed with Soluneem was traced in the xylem vessel up to a height of 6.3 m within 24 h. In an earlier study, an aqueous solution of methylene blue reached a height of 7.0 m in 48 h (ref. 5). During the entire study period, neither button shedding nor yellowing, drooping or drying of leaves was observed in Soluneem-treated trees.

Thirty-year-old coconut trees ($n = 6$) infested with bhc were selected to study the efficacy of Soluneem in the control of bhc. In an earlier study, aerial application of Soluneem at 10 ppm dilution was found to be very effective in the control of pests associated with vegetables, rice and roses. Considering the total biomass of a single coconut tree, 3 bhc-infested trees were systemically administered each with 3000 ppm of Soluneem in 10 ml water while similar infested trees ($n = 3$) were given 10 ml of water only. In order to record the bhc population prior to treatment and at different time intervals from each of the treated and control tree, 25 infested leaflets were clipped from the middle portion of the leaves of 1st, 2nd and 3rd whorls of the palm[6]. Observations were also made on such parameters as per cent pupation, average pupal weight, per cent eclosion and malformation. Total leaf area fed in the damaged leaflet after pupation was recorded from the treatments. The trees were continuously monitored for 12 weeks. Residual analysis of the coconut water for azadirachtin A was carried out by HPLC.

Assessment of bhc population from all the treatments at the time of Soluneem administration revealed majority of the larvae in second instar (78%). The mean bhc population at the commencement of the experiment in both the control and experimental groups were comparable (Table 1). Fifteen days after the treatment, control larval groups were found larger and more active when compared with larvae in the Soluneem group. The population assessment in terms of number of larvae per leaflet showed a declining trend in Soluneem-treated palms from 17th day after administration compared to the control (Table 1). Till the end of the study, the shrunken carcasses of dead larvae were observed in treated palms. The total area fed in the untreated palm leaflets (152 cm^2) were significantly ($P < 0.01$)

Table 1. Larval population in bhc-infested coconut leaflets collected at different time intervals after systemic application of Soluneem

No. of days after application	Larval count (mean ± SD)		*t* value (*t* crit.)	Treatment significance
	Control	Soluneem		
0	5.45 ± 3.06	5.80 ± 2.61	0.407 (1.68)	NS
12	4.24 ± 2.09	3.66 ± 1.68	0.974 (1.68)	NS
47	5.00 ± 1.52	4.16 ± 1.33	2.026 (1.67)	$P < (0.05)$
69	4.60 ± 1.74 (75)*	2.63 ± 1.43 (14.0)*	4.124 (1.68)	$P < (0.01)$
76	3.95 ± 1.67 (44)*	1.90 ± 1.3 (4.0)*	4.466 (1.68)	$P < (0.01)$
110	3.00 ± 1.15	0.72 ± 0.78	5.317 (1.72)	$P < (0.01)$
121	2.52 ± 2.48	0.60 ± 0.78	3.528 (1.67)	$P < (0.01)$

*Numbers in parentheses indicate % adult emergence.

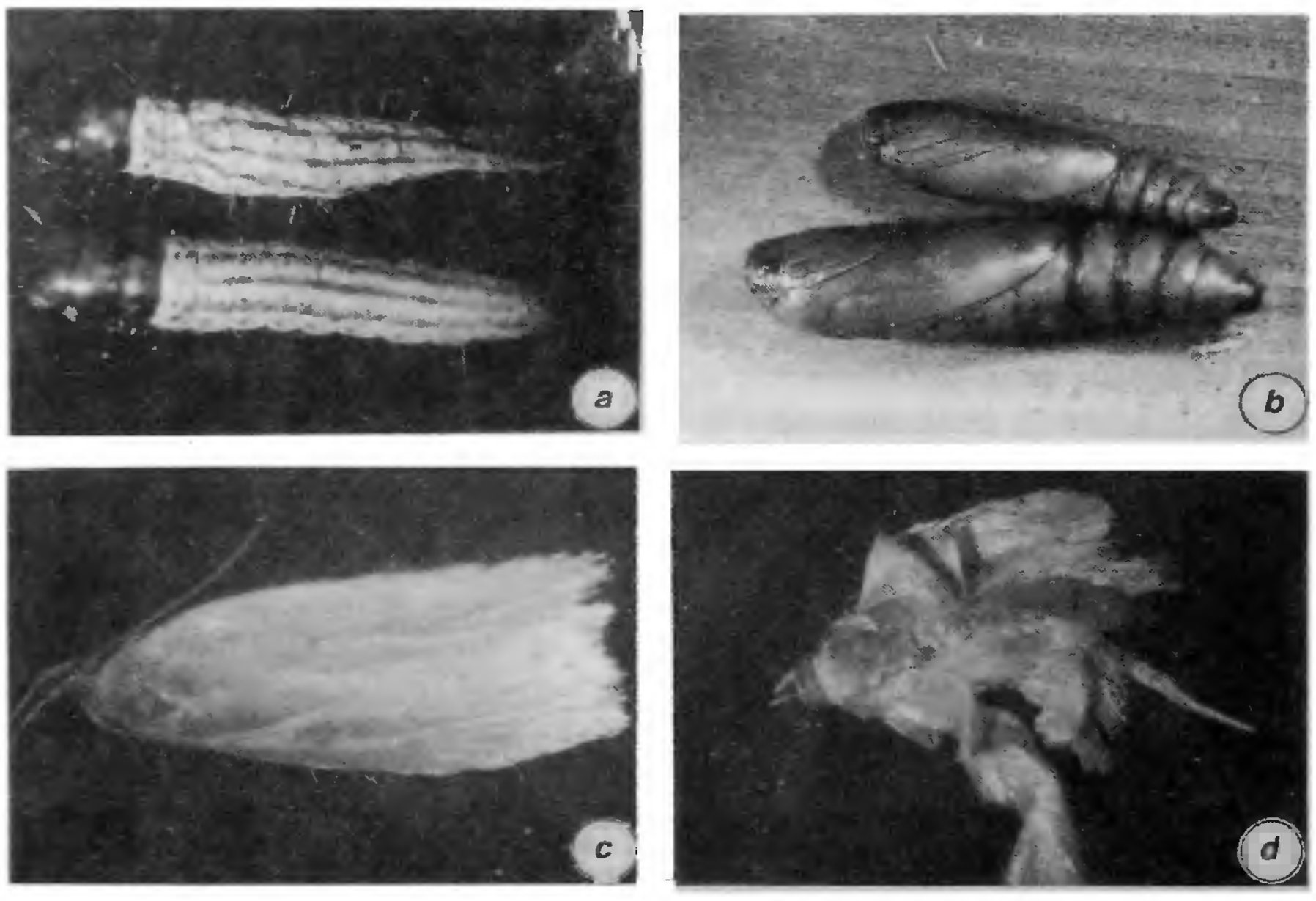

Figure 1. Effect of Soluneem on the larvae, pupae and adults of bhc (*O. arenosella*). *a*, Soluneem-treated malformed (upper) and normal (lower) 5th instar larvae; *b*, Soluneem-treated (upper) and normal (lower) pupae; *c*, normal and *d*, Soluneem-treated malformed adult moths (magnification 8 ×).

Table 2. Effect of Soluneem on the pupal weight of bhc

No. of days after application	No. of observations	Average pupal wt. (g)		*t* value ($P < 0.01$)
		Control	Soluneem	
69	13	0.047 ± 0.008	0.029 ± 0.009	5.58
76	40	0.041 ± 0.011	0.016 ± 0.010	9.88

($t = 4.14$) higher than in the Soluneem-treated leaflets (57 cm^2). The reduction in the feeding efficiency of Soluneem treatment could be attributed to the presence of the insecticide in the leaflets, which contributed to a reduction in physical fitness of the larvae. Reduced feeding efficiency by insects has been reported in a number of instances where a foliar application of a neem-based formulation was used[7]. Throughout the study period, the organoleptic tests of tender coconut showed no change in taste. HPLC analysis of coconut water from nuts harvested from Soluneem-treated trees did not reveal any detectable levels of azadirachtin-A. To the best of our knowledge, this is the first report of systemic application of an azadirachtin containing water-soluble neem insecticide in coconut palms that con-

tributed for reduced feeding and consequent reduction in larval population. Another interesting observation was the delayed pupation as a consequence of extended postembryonic development. By 69th day, only 22% of the larval population on Soluneem-treated palms pupated when compared to 38% in the untreated group. Such delayed post-embryonic development has been reported in several insects upon foliar application of neem-based formulations[8]. A significant ($P < 0.01$) reduction in the average pupal weight was also observed in Soluneem treatment (Table 2) presumably due to reduced feeding on leaflets from Soluneem-treated palms. The most striking observation was the drastic reduction in adult eclosion as a consequence of the treatment (Table 1). A majority of the moths emerging from treated palms (66%) showed malformation, while all the control group pupae emerged into normal adults (Figure 1). An incidence of a fresh early instar larval population of the next generation was observed in both control and Soluneem-treated palms 110 days after initial systemic application (Table 1). However, a significantly ($P < 0.01$) lower number of larvae were recorded on leaflets of Soluneem-treated trees, which could be due to its extended protective effect.

Preliminary observations on the systemic application of Soluneem to coconut trees infested with the eriophyid mite, *Aceria (Eriophyes) guerreronis* have shown that it drastically reduces the population of these mites, which are spreading at an alarming rate in the southern parts of India threatening the coconut industry. In the light of the recent ban on the application of the synthetic pesticide, Monocrotophos, Soluneem could be a safe alternative for the control of pests infesting not only coconut palms but also other economically important perennial trees by systemic application.

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ACKNOWLEDGEMENT. We thank Mr C. N. Puttaswamy for his kind cooperation and permitting us to carry out the studies in his coconut farm.

Received 17 December 1999; revised accepted 6 January 2000

Induction of subcellular malic dehydrogenase activity in fat body cells of diapausing pupae of wild tasar silkworm *Antheraea mylitta* Drury (Lepidoptera: Saturniidae) by 17-β estradiol

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For elucidation of vertebrate estrogen-induced responsiveness of fat body cells of insects, various doses (0.5, 1.0, 5.0, 10 and 50 µg) of 17-β estradiol (E_2) were injected thrice, at 5 day intervals, to 130-day-old diapausing pupae of *Antheraea mylitta* and the changes in cytosolic $NADP^+$-dependent (cMDH, EC 1.1.1.40) and mitochondrial NAD^+-dependent (mMDH, EC 1.1.1.37) malic enzyme activity together with protein content of the subcellular fractions was determined. $NADP^+$-linked cMDH activity decreased in both the sexes at the doses of 10 and 50 µg of estrogen (E_2) per pupa. Lower doses of E_2 (0.5–5 µg) also showed a trend in reduction of specific activity of the enzyme although the data was not found to be significant statistically.

On the contrary, specific activity of NAD^+-linked mMDH was enhanced with all the doses of estrogen used so far in both male and female insects. Protein content in tissue fractions of fat body cells of male and female pupae was elevated with all the doses of injected E_2 when compared to control. In general, females contained more protein in both the tissue fractions than their male counterparts while in case of malic enzyme activity it was found to be just the reverse.

Hence, the activity of $NADP^+$ cMDH and NAD^+ mMDH in fat body cells and its responsiveness to the biologically active hormone estrogen in tasar silkworm has been documented as has been found in *B. mori* and other invertebrates. The steroid hormone effect in diapause regulatory mechanism is also established.

INACTIVATION of prothoracic glands (Pgls) leading to a deficiency of ecdysteroids is the main cause of pupal diapause in holometabolous insects[1,2]. The pupal brain stops releasing PTTH in response to diapause programming signals, and hence inhibits the production of ecdysone from the Pgls, thereby interrupting the pupal–

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