

spheres (figure 4), ooplasm undergoing necrosis, splitting and separation from the ovariole wall and irregular nature of chorionated oocytes. The ooplasm of immature and mature oocytes of ligatured and treated moths showed very faint affinity for ponceau xylydine-acid fuchsin and light green stains.

The structural aberrations in the architecture of ovary induced by ligature and by benzyloxy compounds are strong enough to inhibit egg laying and it has been reported earlier<sup>5</sup> that the ligatured and treated moths do not lay eggs. The common aberrations produced after ligature and chemical treatments suggest the causal factor to be endocrine in nature.

The anatomical as well as histomorphological disorders developed after treatment of benzyloxy compounds are very significant and well related with the reproduction of the insect, and are in agreement, in a general way, with those produced after treatment of terpenoid or sesquiterpenoid juvenoids on many other insects<sup>6-12</sup>. This may be possible because these benzyloxy compounds are designated as potent insect juvenile hormone mimics<sup>2</sup> and the functional principle of these benzyloxy compounds is similar to bio-analogues of juvenile hormone (JH)<sup>13,14</sup>.

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#### COMPARATIVE STUDIES ON GROWTH AND NITROGENASE ACTIVITY OF WATER FERN *AZOLLA* GERMPLASM COLLECTIONS

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THE water fern *Azolla* harbours a symbiotic N<sub>2</sub>-fixing blue-green alga *Anabaena azollae* on its dorsal leaves<sup>1-5</sup>. Species of *Azolla* are widely distributed in both temperate and tropical freshwater ecosystem, although *Azolla pinnata* is commonly found in India. The high rate of nitrogen fixation by *Azolla*, its suitability as a biofertilizer to rice crop, besides its ability to control weed and prevention of water losses, have been recognized<sup>1-4,6-8</sup>. This Institute has collected 86 *Azolla* of different countries belonging to all the seven species. Of these, 48 were found to grow well at Cuttack and among these, 22 belonged to *A. pinnata*, 12 to *A. caroliniana*, 6 to *A. filiculoides*, 2 to *A. microphylla*, 3 to *A. mexicana*, 2 to *A. nilotica* and 1 to *A. rubra*. It was necessary to study their performance to find out better species/strains for practical application in the country. Hence, in the present study, the growth, total chlorophyll and nitrogenase activity (N<sub>2</sub>-fixation) of these collections were determined, when grown in net house and field conditions.

*Azolla* species were cultivated in triplicates in shallow earthen pots (10 cm height × 28 cm diameter) containing flooded soil (3 kg CRRI farm

**Table 1** Nitrogenase activity (nmol ethylene/mg Chl./min), fresh weight (g/pot) and total chlorophyll (mg Chl./g fresh wt.) of *Azolla* species under net house conditions after 25 days of incubation

CRR I accession no	Species collection no	Country	Fresh weight	Total chlorophyll	Nitrogenase activity
<i>A. pinnata</i>					
1	APIA-1	India	85.0	0.50	19.92
2	APIA-2	India	53.0	0.46	16.76
3	APIA-3	India	100.5	0.55	12.57
4	APIA-4	India	86.5	0.42	11.52
5	APIA-5	India	105.5	0.73	16.76
6	APIA-6	India	111.0	0.39	23.05
7	APIA-7	India	107.0	0.36	27.24
8	APNL-1-66	Nepal	91.5	0.46	23.05
9	APNL-11-66	Nepal	108.0	0.59	19.38
10	APNL-111-66	Nepal	129.0	0.42	16.76
11	APPS-55	Philippines	88.0	0.37	15.19
12	APIA-57	Indonesia	111.0	0.50	25.14
13	APTD-59	Thailand	72.0	0.41	20.29
14	APVM-70	Vietnam	90.5	0.56	19.64
15	APBH-64	Bangladesh	80.0	0.46	26.72
16	R-51	Philippines	89.5	0.69	20.95
17	R-52	Malasia	105.0	0.75	17.81
18	R-53	Indonesia	74.0	0.60	18.86
19	IEPI-1	Indonesia	58.0	0.37	24.09
20	IEPI-4	Indonesia	102.5	0.73	31.43
21	IEPI-7	Indonesia	59.5	0.46	23.05
22	SLPI-2	Sri Lanka	53.5	0.34	9.43
<i>A. caroliniana</i>					
101	R-100	USA	116.0	0.62	18.33
102	BLCC-3	Brazil	102.0	0.36	24.09
103	BLCC-5	Brazil	73.0	0.37	23.05
104	BLCC-18	Brazil	108.0	0.63	19.90
105	BLCC-20	Brazil	123.5	0.61	24.10
106	BLCC-21	Brazil	126.5	0.61	28.19
107	BLCC-22	Brazil	109.5	0.62	27.24
108	BLCC-26	Brazil	124.0	0.62	28.19
109	BLCC-28	Brazil	119.0	0.55	20.34
110	UYCC-2	Uruguay	83.0	0.68	20.95
111	WT-V	-	114.0	0.63	22.33
112	RM-PC	-	90.6	0.60	24.57
<i>A. filiculoides</i>					
151	R-10B	USA	88.0	0.62	14.66
152	R-10T	USA	70.0	0.65	29.81
153	R-23	USA	111.0	0.68	27.76
154	R-94	Italy	87.5	0.78	20.43
155	R-95	Italy	81.0	0.73	31.19
156	PUFF-1	Peru	117.0	0.40	24.09
<i>A. microphylla</i>					
201	R-24	USA	58.0	0.55	15.71
202	GSMI-1	Equador	110.0	0.65	30.38

(Contd. . . . next page)

(Table 1 contd.)

<i>A. mexicana</i>					
251	R-54	Guyana	113.5	0.81	23.05
252	R-55	Guyana	130.5	0.84	32.48
253	USMM-2	USA	124.5	0.55	24.57
254	AMUA-201	Philippines	-	-	-
<i>A. nilotica</i>					
276	R-99	Sudan	54.0	0.57	19.90
277	SNMN-1	Sudan	56.0	0.55	9.43
<i>A. rubra</i>					
301	JNRR-1	Japan	83.5	0.56	19.45
C. D (5%)			6.2	0.09	5.6

Inocula 5 g/pot. Inocula was deducted from total fresh weight.

Tentative accession numbers are assigned according to the following system: 1-100 *A. pinnata*; 101-150 *A. caroliniana*; 151-200 *A. filiculoides*; 201-250 *A. microphylla*; 251-275 *A. mexicana*; 276-300 *A. nilotica*; 301-325 *A. rubra*. Collection nos. mentioned in column 2 are given by the concerned Institutions of various countries.

Accession nos. 1-7 local collection, 7-15, 254 from Dr I. Watanabe, IRRI, Philippines; 16, 17, 18, 101, 151-155, 201, 252, 276, from Dr D. W. Rains, University of California, Davis, USA; 19-22, 102-110, 156, 202, 253, 277, 301 from Dr T. A. Lumpkin, Washington State University, Pullman, USA and 111, 112 from Dr G. A. Peters, Battelle-Kettering Research Laboratory, Ohio, USA.

soil pot) with 10 kg P<sub>2</sub>O<sub>5</sub>/ha (applied in three equal splits) under net house conditions, during January to March. The details of *Azolla* collections are given in table 1. Some of the collections (4 of *A. pinnata*, 3 of *A. caroliniana*, 1 each of *A. microphylla* and *A. filiculoides*) were grown in fallow flooded rice fields from November to January. These were multiplied in 2 × 3 m<sup>2</sup> plots in triplicates with 500 g fresh *Azolla* fronds/plot inocula and 10 kg P<sub>2</sub>O<sub>5</sub>/ha. After 25 days of incubation, the biomass (fresh weight), total chlorophyll and nitrogenase activity were estimated. Nitrogenase activity was determined by acetylene reduction technique<sup>9</sup> and total chlorophyll was estimated by the method of Yoshida *et al.*<sup>10</sup>

The growth, nitrogenase activity and total chlorophyll estimation under net house conditions of 48 *Azolla* collection are given in table 1. The *Azolla* species exhibited wide variability in fresh weight, total chlorophyll and nitrogenase activity which varied from 53.0 to 130.5 g/pot, 0.34 to 0.84 mg Chl./g fresh *Azolla* and 9.4 to 32.5 nmol ethylene/mg Chl./min respectively. One of the isolates of *A. mexicana* (no. 252) recorded maximum fresh weight, chlorophyll content and nitrogenase activity, among all the collections. However, another isolate of *A. mexicana* (no. 253) and one isolate each of *A. pinnata* (no. 10) and *A. caroliniana* (no. 106) were comparable to *A. mexicana* (no. 252) with respect to fresh biomass. The nitrogenase activities

in accession nos. 7 and 20 of *A. pinnata*, 106, 107 and 108 of *A. caroliniana*, 152, 153 and 155 of *A. filiculoides* and 202 of *A. microphylla* were at par with the activity in no. 252 of *A. mexicana*. Accession no. 10 was significantly superior to the remaining isolates of *A. pinnata* as regards fresh weight, while nitrogenase activity was maximum in accession no. 20. The nitrogenase activities in accession nos. 8, 7 and 15 were comparable to that in accession no. 20. Among *A. caroliniana*, accession no. 106 showed maximum fresh weight and nitrogenase activity, but fresh weight of this isolate was not significantly greater than those of accession nos. 105 and 108. The nitrogenase activities in accession nos. 102, 103, 105, 107, 108 and 112 were comparable to that in accession no. 106. The chlorophyll contents in accession nos. 102 and 103 were lower than those in other isolates of *A. caroliniana*. The fresh weight of accession nos. 153 and 156 were significantly greater than those of other isolates of *A. filiculoides*, while accession no. 155 exhibited maximum nitrogenase activity. However, the activities in accession nos. 152 and 153 were comparable to that in no. 155. The chlorophyll content of accession no. 156 was significantly lower than those of other isolates of *A. filiculoides*. In *A. microphylla*, accession no. 202 was superior to no. 201 as regards fresh weight, chlorophyll and nitrogenase activity. Accession nos. 276 and 277 of

*A. nilotica* recorded comparable fresh weight and chlorophyll, but the nitrogenase activity in no. 276 was significantly higher than that in no. 277. The *A. microphylla* and *A. nilotica* showed vertical growth unlike other species. Sporulation was observed under net house only in *A. pinnata* isolates (nos. 1–22) and *A. mexicana* (no. 254) during the period January to March.

The growth (fresh weight), total chlorophyll and nitrogenase activity of 4 isolates of *A. pinnata* (nos. 1, 13, 14, 15), 3 isolates of *A. caroliniana* (nos. 102, 104 of *A. caroliniana* were comparable. The 201) and *A. filiculoides* (no. 152) under field conditions are given in figure 1. *A. caroliniana* (nos. 102 and 104) recorded significantly greater fresh weight than the isolates of other species; however, fresh weights of accession nos. 15 of *A. pinnata* and 104 of *A. caroliniana* were comparable. The nitrogenase activity in *A. filiculoides* (no. 152) was significantly higher than those in all isolates of *A. pinnata*, whereas the isolates of *A. caroliniana* and *A. microphylla* were comparable to *A. filiculoides* as regards nitrogenase activity. Among *A. pinnata*, isolate nos. 1 and 14 recorded lower nitrogenase activity than isolate no. 15. Chlorophyll was maximum in isolate no. 112 of *A. caroliniana*; however, it was not significantly greater than those in no. 14 of *A. pinnata*, no. 104 of *A. caroliniana* and no. 201 of

*A. microphylla*. *A. caroliniana* and *A. microphylla* were less susceptible to pests and diseases.

Peters and Mayne<sup>11</sup> reported the nitrogenase activity of 25–30 nmol ethylene/mg Chl./min in *A. caroliniana* grown under controlled conditions. The nitrogenase activity of *A. mexicana* was 29.9 nmol ethylene/mg Chl./min in mineral medium under net house conditions<sup>12</sup>. *A. mexicana* showed comparatively better enzyme activity in this study. Among isolates of *A. pinnata*, an isolate of Indonesia (no. 20) exhibited highest nitrogenase activity (31.43 nmol ethylene/mg Chl./min) under net house conditions. The growth of seven isolates of *A. pinnata* and one each of *A. filiculoides* and *A. mexicana* were studied earlier under field conditions where the Thailand isolate of *A. pinnata* was reported to be better than others<sup>3,6</sup>. In the present study, *A. caroliniana* (no. 102) showed better growth than others and among *A. pinnata* isolates, no. 15 (Bangladesh) produced maximum biomass under field condition, although *A. filiculoides* exhibited maximum nitrogenase activity. *A. pinnata* is reported to grow well throughout the year in fields at Cuttack, whereas *A. filiculoides* and *A. mexicana* grew well only during winter season<sup>6</sup>. It is difficult to compare growth and nitrogenase activity in different *Azolla* species from various reports because of the difference in growth conditions<sup>5</sup>. The

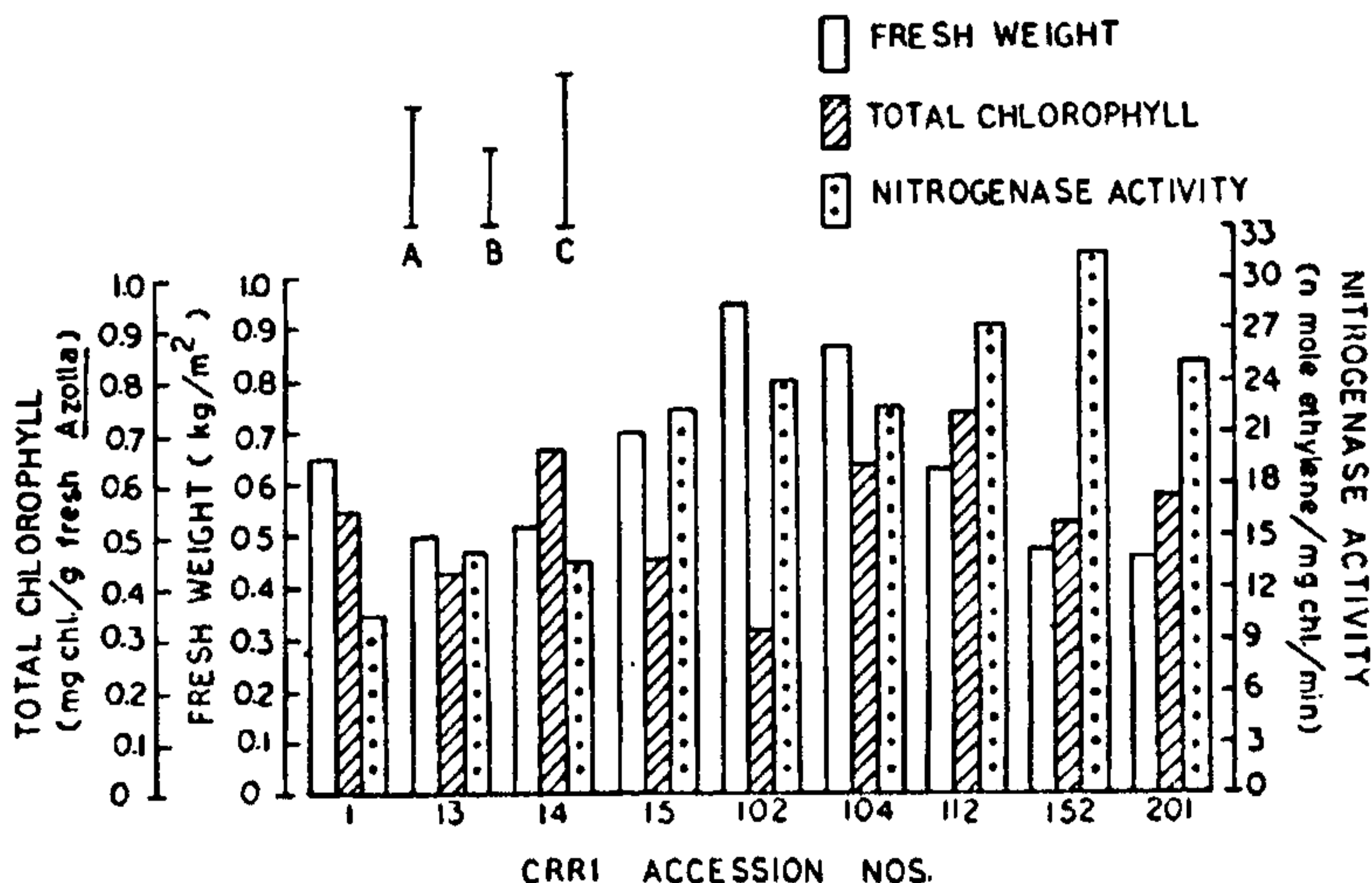


Figure 1. Nitrogenase activity, growth and total chlorophyll of *Azolla* collections under field conditions after 25 days of incubation. Bars indicate the critical difference (C.D.) at 5% probability level for comparing fresh weight (A), total chlorophyll (B) and nitrogenase activity (C).

frequent variation in pigmentation occurs in *Azolla* under field conditions according to light intensity, temperature and nutrient availability except *A. pinnata* isolate from Vietnam (no. 14) which remained green when water temperature was high during summer and was also found to tolerate low temperature at Cuttack<sup>13</sup>. The sporophyte of *Azolla* is heterosporous, since both mega and microsporocarps are produced on the same plant, except in *A. pinnata* green isolate where only microsporocarps are formed<sup>2,14</sup>. Formation of sporocarps in *A. pinnata* isolates, *A. filiculoides* and *A. mexicana* is reported at Cuttack conditions where the latter species sporulate throughout the year<sup>3</sup>. The present observations show that all the species could grow in net house conditions; however, only *A. pinnata* (nos. 1–22) and *A. mexicana* (no. 254) sporulated during the period of experimentation. It is necessary to screen *Azolla* resistant/tolerant species/varieties for its pests. Singh<sup>3</sup> reported Bangladesh isolate of *A. pinnata* as tolerant to pests in comparison to other isolates. The success of *A. caroliniana* multiplication throughout the year in field is perhaps due to its less susceptibility to pests including snails and diseases besides its tolerance to high and low temperatures.

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**A NEW HOST RECORD OF A HYPERPARASITE, PERILAMPUS SP. (PERILAMPIDAE : HYMENOPTERA) ON APANTELES ARISTAEUS (NIXON.) (BRACONIDAE : HYMENOPTERA)**

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TEA is made from the young leaves and unopened buds of the tea-plants, *Camellia sinensis* (L.) O. Kuntze<sup>1</sup>. A number of pests cause considerable damage to these plants<sup>2,3</sup>. The insect pest causing heavy economic loss is the larva of the moth, *Cydia leucostoma* (Meyr.)<sup>4</sup>. The larvae are usually called 'flushworms'. They were parasitized by a number of insect parasites<sup>5</sup>. *Perilampus* sp. (tristis group) was the hyperparasite of flushworm and larval parasite of *Bracon* sp.<sup>5</sup>. The present investigation revealed that the *Perilampus* is also a larval parasite on *Apanteles aristaeus* (Nixon.). The presence of *Perilampus* on *A. aristaeus* is a new host record.

Several hundreds of flushworm-infested shoots were collected in and around Kil-Kotagiri, Nilgiris, Tamil Nadu (4800' to 5500' above MSL) throughout the years from 1982 to 1986. The pest larvae were separated from the infested shoots and reared in transparent plastic containers. The normal larva is pinkish or brownish (figure 1) while the infected one has a translucent body with a conspicuous longitudinal red mark middorsally (figure 2). The latter ones