found defoliating pines and is considered a potential pest of tropical pines in India[3]. On mango it is recorded from the Philippines[4]. In addition, the authors observed the pest feeding on *Eugenia jambolana* and pear.

The young larvae feed on new flush, also nibbling the leaf and shoot buds and affecting growing points. The later stages feed on leaves and nibble new soft shoots. In case of severe attack, fruit stalks and fruits were also scraped (figure 1), resulting in drying up of affected tissues and rendering the fruits unmarketable.

The female moth, often with under-developed wings or wingless (figure 2), lays eggs in groups (number varied from 2 to 85 in the laboratory). Larval development takes 3–4 weeks. The full-grown larva is a light-brown hairy caterpillar about 3 to 3.5 cm in length with orange-red mouth and creamish-yellow and brown tufts of hairs (figure 4). Pupation takes place on the plant in a thin, papery cocoon covered with larval hairs. Freshly formed pupa appears creamish-white and changes to light brown. The female pupa is bigger. Pupal period is 7 to 10 days. Caterpillars were seen defoliating mango in Lucknow from April to August in 1987.

Larval parasite *Exorista* sp. (Diptera: Tachinidae) and pupal parasite *Brachymeria lasus* Walker (Hymenoptera: Chalcididae) were recorded on this pest from Lucknow.

In view of the severe damage the pest can cause, it may be considered as a potential pest in mango orchards of Uttar Pradesh and other regions of the country.

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1. Beeson, C. F. C., *Ecology and Control of Forest Insects of India and Adjacent Countries*, Vasant Press, Dehradun, 1941, p. 1007.
2. Nair, M. R. G. K., *Insects and Mites of Crops in India*, Indian Council of Agricultural Research, New Delhi, 1975, p. 404.
3. Singh, Pratap, Fasih, M. and Prasad, G., *Indian For.*, 1982, **108**, 93.
4 Tandon, P. L. and Verghese, A., *World List of Insects, Mites and Other Pests of Mango*, Tech. Doc. No. 5, IIHR, Bangalore, 1985, p. 22.

HARVESTING HIGH CELL DENSITIES FOR DEVELOPMENT OF *RHIZOBIUM* CELL CONCENTRATE

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A high number of viable rhizobia in inoculants is vitally important for successful use of the inoculants. Commercial inoculants, however, contain not more than 10^9 viable rhizobia per gram of wet carrier at manufacture[1]. Besides, their population declines rapidly with time, especially when inoculants are exposed to high temperature during storage and transportation, and render the inoculants ineffective. Most of the time it becomes difficult to maintain even the recommended minimum number of viable rhizobia in carrier during prolonged storage of inoculant. Stocks of cell concentrates of *Rhizobium* may overcome this problem to a great extent. They may be prepared well in advance to meet any unprecedented seasonal demand. Any type of (seed of soil) inoculants of required standard may be prepared from them just before use.

Frozen cell concentrates of *Rhizobium* strains have been used in the preparation of inoculants, particularly in North America[2]. These concentrates are easy to store and transport as they occupy approximately 300 times less space than carrier-based inoculants. They can easily be preserved either by freeze-drying or in 40% glycerol for prolonged periods[3]. In spite of many advantages, cell concentrates of *Rhizobium* are not very popular. The main reason for this is the low recovery of viable rhizobia from growth medium, which not only affects the efficiency but also the cost of production.

In this study, we assessed the potential of various growth substrates for harvesting high cell densities of *Rhizobium* from the growth medium.

Yeast extract mannitol (YEM) broth[4] was used as standard medium for comparison in all the growth experiments. However, in some experiments, mannitol, molasses, malt extract or jaggery was added (1 g) in place of 10 g per litre of mannitol. In experiments where a common source of carbon and nitrogen was used, yeast extract and malt extract were added separately (2, 6 and 10 g/l). Cultures of *Rhizobium leguminosarum* (P-3-86) and *Bradyrhizobium japonicum* (SB-16) were grown at 30±2°C for 72 h on a rotary shaker (120 rpm). The cells were recovered from the medium by centrifugation at

7000 *g* for 20 min. The cells were resuspended in sterilized phosphate buffer (pH 7.2). The number of viable rhizobia in suspension was enumerated by plate count using a standard yeast extract mannitol agar medium containing congo red[4].

Rhizobium species are known to produce polysaccharides from sugars in growth medium[5]. This results in higher viscosity of culture medium and leads to inefficient separation of cells by centrifugation[6]. In YEM broth, though it supports good

Table 1 *Growth yields and yield recoveries of Rhizobium leguminosarum and Bradyrhizobium japonicum on different growth substrates in liquid medium*

		R. leguminosarum (P-3-86)		*B. japonicum* (SB-16)	
Nutrient	Conc. (%)	Growth yield (log cfu/ml)	Yield recovery (%)	Growth yield (log cfu/ml)	Yield recovery (%)
Mannitol	1.0	10.25	15.18 (22.87)	10.16	15.25 (22.98)
Mannitol	0.1	9.09	25.56 (30.37)	8.28	31.38 (34.08)
Molasses	0.1	8.62	28.05 (31.97)	8.56	40.69 (39.64)
Malt extract	0.1	8.10	71.00 (57.42)	7.44	72.53 (58.37)
Jaggery	0.1	8.39	32.58 (34.82)	8.59	44.84 (42.02)
		Growth yield		Yield recovery	
SE(±)		0.04		(3.78)	
CD at 5%		0.12		(11.34)	

Figures in parentheses are angular transformation values.

Table 2 *Influence of substrate concentration on growth yield and yield recoveries of Rhizobium leguminosarum and Bradyrhizobium japonicum in liquid medium*

		R. leguminosarum (P-3-86)		*B. japonicum* (SB-16)	
	Conc. (%)	Growth yield (log cfu/ml)	Yield recovery (%)	Growth yield (log cfu/ml)	Yield recovery (%)
Malt extract					
	0.2	9.03	72.18 (58.18)	7.56	72.00 (58.05)
	0.6	9.31	65.22 (53.85)	8.16	70.82 (57.29)
	1.0	9.79	62.54 (52.24)	8.70	61.48 (51.65)
Yeast extract					
	0.2	9.65	71.81 (57.81)	8.14	70.82 (57.29)
	0.6	10.20	70.85 (57.35)	9.29	68.69 (55.98)
	1.0	9.32	72.00 (58.05)	9.00	68.14 (55.61)
		Growth yield		Yield recovery	
SE(±)		0.04		(3.75)	
CD at 5%		0.12		(11.25)	

Figures in parentheses are angular transformation values.

growth of rhizobia, the recovery of viable rhizobia is very low (less than 1%)[5]. The results of this study also show low recovery of viable rhizobia from standard YEM broth on centrifugation (table 1). Improvement was observed when mannitol was used at suboptimal level (1 g/l) in the growth medium, but the results are much better with malt extract, which gave 71–72.53% recovery of viable rhizobia in comparison to 15.18–15.25% with mannitol. Malt extract, when used as sole source of carbon, nitrogen and growth factors in the medium, was found to be better than yeast extract which has been reported to be very good for growth yield and yield recovery of *R. leguminosarum*[5]. It was, however, true only for lower concentration (2 g/l) of malt extract in liquid medium (table 2). At higher concentrations (6–10 g/l) the recovery of viable rhizobia declined, though growth yield improved. With yeast extract, however, recovery yields were good even at higher concentration.

It may be inferred from this study that a large population of viable rhizobia may be recovered from the growth medium for developing cell concentrates if malt extract is used in lower concentration as sole source of carbon, nitrogen and growth factors in the medium. This will not only reduce the cost of production (as malt extract is cheaper than yeast extract) but also improve the efficiency of production of cell concentrates because of faster recovery of cells from growth medium.

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1. Thomson, J. A., In: *Methods for Evaluating Biological Nitrogen Fixation*, (ed.) F. J. Bergerson, John Wiley and Sons, New York, 1980, p. 489.
2. Portor, F. E., In: *Developments in Industrial Microbiology*, American Institute of Biological Sciences, Washington, 1969, vol. 10, p. 88.
3. Meade, J., Higgins, P. and O'Gara, F., *J. Appl. Bacteriol.*, 1985, 58, 517.
4. Vincent, J. M., *A Manual for the Practical Study of Root-Nodule Bacteria*, Blackwell Scientific Publications, Oxford, 1970.
5. Ghai, S. K., Hisamatsu, M., Amenura, A. and Harada, T., *J. Gen. Microbiol.*, 1981, **122**, 33.
6. Elsworth, R., *Phase Separation of Suspensions by Centrifuge*, New Brunswick Sci. Co., New Jersey, 1962.

REVERSAL OF CHLORAMPHENICOL-INHIBITED PROTEIN SYNTHESIS BY EDTA AND SULPHHYDRYL COMPOUNDS IN BHENDI SEEDLINGS (*ABELMOSCHUS ESCULENTUS* (L.) MOENCH.)

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It is well established that chloramphenicol inhibits protein synthesis on 70 S ribosomes[1]. Attempts have been made to use this inhibitor to clarify characteristics of protein synthesis in higher plants[2–4]. While chloroplast and mitochondrial ribosomes bind chloramphenicol, cycloheximide blocks protein synthesis on cytoplasmic 80 S ribosomes[5,6]. This communication presents results of experiments to test the ability of EDTA and three sulphhydryl compounds to reverse the effect of chloramphenicol in bhendi seedlings. EDTA is a metal-chelating agent and can conjugate protein[7]. Chemicals like glutathione, cysteine, ascorbic acid and thiourea have been shown to markedly reduce chromosomal aberrations caused by gamma radiation in *Tradescantia* and onion root tips[8,9]. The stability of the disulphide bond makes it an important factor in the tertiary structure of proteins[10]. Sulphhydryl groups and sulphydryl-binding reagents can participate in redox reactions by reversible formation of disulphide bonds.

Seeds of bhendi var. Pusa Savani were surface-sterilized with 5% mercuric chloride for 15 min and washed thoroughly with distilled water. They were then soaked before sowing for 24 h in 200 ppm solutions of EDTA, methionine, cysteine, glutathione or chloramphenicol or combinations of these compounds (see figure 1). Lower concentrations (50 and 100 ppm) were ineffective. Seedlings were allowed to grow in distilled water for 10 days under a light intensity of 2000 $\mu E\, m^{-2}\, sec^{-1}$. Seedling protein was extracted[11] and estimated[12] as described earlier. Increase or decrease in protein was calculated as per cent difference between control and treated seeds.

The results (figure 1) indicate that in EDTA-treated seedlings there was about 19.7% increase in protein content on day 10. Chloramphenicol treatment caused a 29.3% reduction in protein content. However, when seeds were treated with EDTA and chloramphenicol, the decrease in protein content was only 4.3% on day 10 of seedling growth. Thus