treatment was replicated thrice. Untreated plants sprayed with distilled water were used as control. Disease rating was made by counting the number of leaf spots per plant.

Daily foliar spray showed significant effects ($P > 0.01$) of cyanobacterial cell extracts in controlling bacterial leaf spots of chilli. Both foliar application tests and laboratory studies proved the superiority of *Lyngbya* cell extract in controlling disease development and growth of the pathogen. Foliar application of 100 mg l^{-1} *Lyngbya* cell extract reduced chilli leaf spots by 70%, whereas for other strains the effectiveness was below 50% (Figure 1). High concentrations were found to be more effective in disease control. At 500 mg l^{-1} of *Lyngbya* cell extract, no foliar spots were observed. At this concentration, for *Microcystis* and *Plectonema*, the effectiveness remained 90%. Laboratory trials also indicated concentration-dependent effects of different cell extracts on growth of *X. vesicatoria*. All the concentrations of the three cyanobacterial extracts significantly reduced the growth of the pathogen (Table 1). Parallel to the foliar spray, no growth of the pathogen was observed at 500 mg l^{-1} of *Lyngbya* cell extract. The study indicated that the cell extracts of all the three cyanobacterial strains have anti-bacterial properties against *X. vesicatoria*; however *Lyngbya* cell extract is most effective. These observations form the first report of antibacterial properties of three cyanobacteria in controlling leaf spot disease of chilli. This may be a cost-effective and eco-friendly approach to control bacterial leaf spot disease of chilli.

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Heparinase in purple fluid of the sea hare, *Bursatella leachii*

The physiological role of heparin has been a subject of considerable research in recent years because of its strong anti-coagulant property and its ability to induce the appearance of lipoprotein lipase in the circulatory system of vertebrates, including man. Heparin may be hydrolysed with acid only under rigorous conditions, which results in considerable caramelization, especially of the glucuronic acid, and extensive splitting of heparin. Thus, the products are mono- or di-saccharides from which all the sulphate groups are removed[1]. Studies on enzymatic hydrolysis and isolation and characterization of partial hydrolysis products, would reveal the fine structure of heparin.

Heparin can induce some bacteria to produce a series of enzymes, including glycuronidase, sulphoesterase and sulphamidase as well as heparinase and heparatinase[2]. Heparinase is an α1-4 eliminase which acts specifically on the glycosidic linkage between N-sulphated D-glucosamine and sulphated D-glucuronic acid (or L-iduronic acid) present in heparin[3]. The elimination reaction at this site, catalysed by heparinase, results in the formation of smaller polysaccharides with both a reducing end and an α,β-unsaturated acid end-group. This enzyme, despite a high demand for it by researchers, has not yet been made commercially available. In this present study, we tried the degradation of heparin by the purple fluid of *Bursatella leachii*.

The purple fluid of *B. leachii* was obtained by irritating the animal. The fluid was centrifuged (4000 rpm in 15 min) and the supernatant was lyophilized and stored at 4°C until it was used for determining the heparinase activity. Two methods were followed and are described below.

In azure-A assay, heparinase activity was determined in terms of decrease in heparin content when mixed with the purple fluid extract. The content of heparin was quantified based on decrease in metachromasia with a reagent, azure-A, with some modifications[4]. Heparin was incubated with the purple fluid of *B. leachii* (1 mg of heparin per ml of fluid) for 1 h at room temperature (28 ± 2°C). Placing the mixture in a water bath at 60°C for 10 min stopped the reaction. Various concentrations of the reaction mixture were drawn and added to 10 ml of 0.02 mg/ml azure-A dye solution. The dye showed a metachromatic shift from blue to red in the presence of heparin. The change in optical density was measured within 1 h at 620 nm and compared with a standard curve and controls. Two controls were maintained – heparin + azure-A as a positive control, and heparin + inactivated sample (by heating at 60°C for 1 h) + azure A as negative control.

The heparinase activity was measured with some modifications[5]. Heparin was incubated with the purple fluid of *B. leachii* (1 mg of heparin per ml of fluid) for 1 h at room temperature (28 ± 2°C), and placing the mixture in a water bath at 60°C for 10 min stopped the reaction. The turbidity was removed by centrifugation and the supernatant solution was

Table 1. Time taken for coagulation of blood treated with heparin and purple fluid (200 mg/ml) of *Bursatella leachii*. (The experiment was conducted four times.)

Time (seconds)	Blood	Heparin + blood	Heparin + blood + inactivated fluid	Heparin + blood + fluid
0	–	–	–	–
150	–	–	–	–
300	–	–	–	–
450	+	–	–	–
600	+	–	–	–
750	+	–	–	–
900	+	–	–	–
1050	+	–	–	–
1200	+	–	–	–
1350	+	–	–	+
1500	+	–	–	+
1650	+	–	–	+

+: Blood clots; –: Blood does not clot.

used for the anticoagulation assay and coagulation time was noted in comparison with controls. Two controls were maintained – normal blood + heparin as a positive control and heparinized blood + inactivated sample (by heating at 60°C for 1 h) as negative control.

To measure toxicity by haemolytic assay, blood (groups A, B and O) was collected from a blood bank. Haemolytic assay was performed[6] to find out the toxic effect of the purple fluid extract of *B. leachii*. Different groups of human RBC (A, B and O), suspended in saline, were incubated separately at 37°C with the purple fluid for 1 h. The mixture was centrifuged and the supernatant was measured for its absorbence at 530 nm using a colorimeter (ERMA AE-11N, India). Lysis of erythrocytes in triple distilled water was considered as 100%.

Protein estimation was followed by modified method of Lowry *et al.*[7] using bovine serum albumin as a standard. This method is applicable to soluble membrane and conjugated proteins in dilute solutions together with removal of most interfering substances.

In the azure-A assay of heparinase, the specific activity of heparinase (1 unit = 1 mg of heparin degraded/mg of protein/h) was measured at 0.0624 mg/mg/h.

The results of the bioassay of heparinase revealed that the purple fluid of *B. leachii* showed the activity against the anticoagulant heparin. For instance, the blood did not exhibit any coagulation when heparin was added. However, when fluid sample was added at 200 µg/ml, the blood showed coagulation after 1350 sec (Table 1).

Haemolytic assay of purple fluid showed that it did not exhibit any lytic activity or cytotoxicity in different human erythrocytes – A, B, O.

The heparinase activity has been reported to occur in preparations of mammalian liver[8–10]. This activity, however, is extremely low and no evidence has been provided as to the nature of the enzymatic process involved. The *B. leachii* extract described in this present study has an enzyme system capable of catalysing the heparin.

Heparin-like anticoagulant is known to occur in marine molluscs – *Busycon undatum*[11], *Katelysia opima*[12], *Anomalacardia brasilina*, *Tivela mactroides* and *Donax strictus*[13]. Though the presence of such anticoagulants in various molluscs has been reported, their exact function in molluscs is still not known. It is not possible for the compound to act as an anticoagulant, since molluscs are devoid of coagulant system as is present in mammals.

Since, heparin is an acid mucopolysaccharide, it binds tightly to many proteins, which in turn changes several of its physical characteristics, including its isoelectric point. Several proteins such as protamines and histones can interfere with its anticoagulant activity[14]. The purple fluid showed activity against the heparin-like activity, but did not exhibit any haemolytic activity.

The sea hare *Aplysia* and *Dolabella* species have been reported to contain some biologically active substances, including antibacterial factors[15], haemagglutins[16,17], toxins[18], cytotoxins[19,20] and chemical defensive substances[21,22]. Most of these substances are of low molecular weight, derived from the algae on which the sea hares feed[22,23]. But, there was no such study on other members of Aplysiomorphna like *Bursatella leachii*, the test organism of the present study. This species discharges a purple fluid from the purple gland when disturbed. This reaction suggests that the fluid may contain bioactive factors which may act against potential enemies since the defence mechanisms of the sea hares are well-developed, comparable to highly-developed vertebrates[24]. Hence, further attempts merit attention on purification and characterization of the purple fluid, because of its high biological activity against the heparinized blood and on the elucidation of physiological functions of this factor.

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A simple and cost-effective mass rearing technique for the tephritid fruit fly, *Bactrocera dorsalis* (Hendel)

Fruit flies belonging to the families Tephritidae and Drosophilidae are major subjects for basic scientific investigations. The genus *Drosophila* of the latter family has been a subject of genetic and molecular studies on account of its easy rearing and quick adaptability to laboratory conditions. According to Wood *et al.*[1], the tephritids also have many qualities of a good genetic subject. Ecologists and geneticists are becoming increasingly aware of the diversity and rapidity of evolutionary changes taking place in sibling species complexes of tephritid fruit flies. This has been a fascinating subject of study with great economic implications, and has led to investigations in genetic studies like genome mapping, on these species. Such studies help to understand the significance of evolutionary changes, biosystematics, ecotypes, and biotechnological and molecular clues as basis for advancing tephritid management. Spanos *et al.*[2] studied the complete sequence of the mitochondrial genome of *Ceratitis capitata* Weidemann, that is potentially useful for the development of diagnostic tools for population analysis applications, as for example, determining the source of new introductions. Orchando and Reyes[3] studied the genetic population structure, gene flow and patterns of geographic distribution in olive fly, *Bactrocera oleae* (Gmelin) and found that natural selection, probably due to agricultural practices, may be the major factor responsible for the pattern of genetic variability observed in that species.

The potential of tephritids, though good laboratory material for investigations, has been under-exploited. One of the main reasons is due to problems related to the easiness of culturing the insects. A pre-requisite for a successful study of the genetics or for management applications like male sterile techniques of these fruit flies, is the improvement of its rearing technique. For this study, we selected the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Tephritidae: Diptera) which has been extensively studied by evolutionary and population geneticists, and its host races and sibling species form a classical example of presumed sympatric speciation[4–6]. It is widely distributed in the Oriental region[6] from Australia and Hawaii to Pakistan; hence it is also called Oriental fruit fly. It has also been reported from California, USA[7] in 1974, but was subsequently eradicated[8]. Further, it is a major pest of mango (*Mangifera indica* L.) in several parts of the world[7].

The artificial diet first recommended for rearing the Oriental fruit fly, *B. dorsalis* consisted of fresh carrots as a major ingredient supplemented with brewer's yeast and inhibitors against mould and bacterial growth[9]. Mitchell *et al.*[10] described a rearing medium for *B. dorsalis*, consisting of dehydrated carrot powder (in the place of fresh carrots) with brewer's yeast, sodium benzoate and hydrochloric acid. Later, Rejesus[11] mass-reared *B. dorsalis* on an artificial diet containing cooked yellow sweet potato (*Ipomea batatus* L.), rice bran, brewer's yeast, sugar, sodium benzoate, water and hydrochloric acid. Tanaka *et al.*[12] developed a low-cost rearing medium for *B. dorsalis* using methyl ρ-hydroxybenzoate, sodium yeast type 200, concentrated hydrochloric acid, wheat shorts, wheat middlings, gelgard M and water. The main problem encountered using carrots in artificial diets is the microbial contamination of diets and consequent high price of ingredients such as brewer's yeast and dehydrated carrot powder. Since the goal of mass rearing is to produce maximum number of healthy insects economically, a need was felt to make an attempt to mass-rear fruit flies on cheaper fruits easily available throughout the year, in countries distributed in the Orient. In any mass rearing, artificial diets are resorted to, only if obtaining and maintenance of natural host is difficult. For example, rearing mealybug on cucurbits with long shelf-life (e.g. pumpkin) is cheaper and easily viable than on arti-