extracts were stable in casein beads at non-toxic level. However, it was found to be unstable in uncapsulated extracts for both the plants. Moreover, the encapsulated extracts at room temperature also showed no significant colour change in beads over a period of 12 months. In conclusion, our present study has commercial potential; we are working with an industrial partner under confidential agreement and the data are promising.

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Using bryophytes as a tool to cure European foulbrood disease of honey bee: an eco-friendly approach

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European foulbrood disease is a broad disease in honey bees caused by a bacterium, *Melissococcus plutonius*. By now, various herbal and chemical drugs have been tried to control it. In the present study, the effects of different organic extracts of three different bryophytes and a standard drug (positive control) have been tried to control the bacterium *in vitro* by using agar disc diffusion and micro broth dilution method. All the tested extracts showed good antibacterial

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activities against the test pathogen. Acetone extract of *M. polymorpha* and chloroform extract of *D. undulatum* exhibited maximum activity (AI 15.51 and 15.56 mm respectively) comparable to that of standard drug.

Keywords: Activity index, bryophytes, European foulbrood, honey bees.

MELISSOCOCCUS PLUTONIUS, the causal agent of European foulbrood (EFB) disease in honey bees, is one of the most dangerous honey-bee pathogens. An antibiotic, oxytetracycline (OTC) is generally used which is ingested by the larvae, where it acts on *M. plutonius* present in the larval gut[1,2]. However, the bacteria, except some isolates, are developing resistance to this antibiotic[3]. Also, OTC residues have been identified in honey up to 6–9 weeks following treatment[4]. It has also been found that the antibiotic reduced disease symptoms more rapidly, but it did not cure EFB in the long term. The problem of residue effects and developing resistance of the bacterium necessitated the use of eco-friendly approaches through the use of several botanicals, viz. neem (*Azadirachta indica*), turmeric (*Curcuma longa*) and cinnamon (*Cinnamomum vernum*) to control the disease[5]. Cow urine has also been tried to control the disease[6]. There is hardly any report on the use of bryophytes, a unique group in the plant kingdom having a rich storehouse of biologically active compounds, to control the diseases of honey bees. In human diseases, bryophytes have been used since time immemorial. They are used in pharmaceutical products, horticulture, for household purposes and are also ecologically important[7]. Extracts of *Ceratodon purpureus* and *Bryum argenteum* have been patented to cure fungal infections in horses[8]. In the present study, an attempt has been made to use bryophytes to control *M. plutonius*.

Four bryophytes, *Marchantia polymorpha* L., *Plagiochasma appendiculatum* L. (liverworts), *Dicranum undulatum* Schrad. ex Brid. and *Isopterygium elegans* (Brid.) Lindb (mosses) were collected from Champawat (29°5′ and 29°30′ in the northern lat. and 79°59′ and 80°3′ at the centre of the eastern long.), Uttarakhand, India in July 2010. Voucher specimens were submitted to the Department of Biological Sciences, G.B. Pant University of Agriculture and Technology (GBPUAT), Pantnagar. The plant material was carefully cleaned from attached litter and dead material, under running tap water and finally with sterile distilled water, shade-dried and then finely powdered (100 g) with the help of a grinder. The powdered plant material was then Soxhlet extracted with 500 ml of five different organic solvents (petroleum ether, methanol, chloroform, ethanol and acetone) for 24 h, filtered, dried *in vacuo* and kept in a refrigerator for further studies. *M. plutonius* was isolated from the midgut of the infected bee using streaking method and used as test pathogen. For antibacterial screening, disc diffusion assay technique was used[9]. Nutrient agar (NA) was distributed in sterilized petri plates. Spread plate method was used for inoculation of bacterial suspension (1×10^8 CFU/ml) prepared in sterilized water. Sterilized filter paper (Whatman No. 1) discs of size 5 mm were individually impregnated with 100 µl of 1 mg/100 µl extracts to obtain 1 mg/disk air-dried under sterilized conditions and placed on the inoculated agar plates along with the standard 1 mg/disk (ampicillin). Inoculated petri plates were incubated at 37°C for 24 h. The diameter of the inhibition zone was measured in millimetres. Antibacterial activity was expressed in terms of activity index (AI).

$$\text{AI} = \text{Inhibition zone of the test sample } (T) / \text{Inhibition zone of the control } (C).$$

The minimum inhibitory concentration (MIC) was determined using the 96-well micro broth dilution method[10]. Stock solutions of extracts were prepared using the respective solvents of extracts. Test solutions were prepared by twofold serial dilution of stock solutions and were added into a 96-well micro titre plate, already containing broth media (NA broth); 50 µl of inoculum suspension (1×10^8 CFU/ml for bacteria) prepared in sterilized water was then added to the wells of the micro titre plates. Cultures containing standard drug solution (ampicillin) were used as positive controls. The MIC was regarded as the lowest concentration able to inhibit any visible bacterial growth. Minimum bactericidal concentration (MBC) was determined by sub-culturing the aliquots of inoculum from the wells. Each extract was tested in triplicate and the experiment was performed three times. Values were represented as mean ± SE ANOVA revealed significant differences between different organic solvents utilizing Dunkan's multiple range test (DMRT)[11].

Organic extracts of all bryophytes, except *I. elegans* exhibited good inhibitory activity against the test pathogen (AI ranging from 8.56 to 15.56; Table 1). The acetone extract of *M. polymorpha* and chloroform extract of *D. undulatum* were found to be the most potent (AI = 15.51 and 15.56 respectively) inhibitors equivalent to the positive control, ampicillin (AI = 15.56). Acetone extract of *M. polymorpha* was found to have an activity equal to that of chloroform extract of *D. undulatum*. Acetone extract of *P. appendiculatum* exhibited maximum AI (13.56), which was lower than the other bryophytes. Aqueous extracts of all the bryophytes were ineffective in controlling bacterial growth. The MIC values ranged from 0.65 to 2.50 µg ml^{-1} (Table 2). For ethanol extract of *M. polymorpha*, the MIC and MBC values were the same (1.50 µg ml^{-1}), whereas in all other cases the MBC values were higher than their respective MIC values suggesting their bactericidal and bacteriostatic nature. A perusal of MIC values of different extracts indicated that the bacterium is highly sensitive to chloroform extract of *D. undulatum* and acetone extracts of *M. polymorpha* and

Table 1. Antibacterial activity of different bryophyte extracts in different solvents (expressed as activity index) against *Melissococcus plutonius*

Extract	*Marchantia polymorpha*	*Plagiochasma appendiculatum*	*Dicranum undulatum*
Petroleum ether	0.0	10.55 ± 0.36	8.56 ± 0.37
Methanol	12.45 ± 0.37[a]	11.43 ± 0.21[a]	10.61 ± 0.35
Chloroform	0.0	0.0	15.56 ± 0.37
Ethanol	13.56 ± 0.25[a]	11.59 ± 0.31[a]	0.0
Acetone	15.51 ± 0.25	13.56 ± 0.38	0.0
Cd	0.599	0.623	0.688
Cv	2.638	2.417	0.626
F value	3666.491*	1674.348*	2206.844*
Positive control			
Ampicillin	15.56 ± 0.37	15.56 ± 0.39	15.56 ± 0.37

The same superscripted alphabets in each column show significant value with each other at $P \geq 0.05$ by DMRT.
*Significant at $P < 0.01$.

Table 2. Minimum inhibition concentration (MIC; μg ml^{-1}) and minimum bactericidal concentration (MBC; μg ml^{-1}) of different organic extracts against *Melissococcus plutonius*

	M. polymorpha		*P. appendiculatum*		*D. undulatum*	
Extract	MIC	MBC	MIC	MBC	MIC	MBC
Petroleum ether	–	–	2.50	4.50	2.50	5.00
Methanol	2.50	3.00	2.50	4.50	1.25	2.50
Chloroform	–	–	–	–	0.65	1.25
Ethanol	1.50	1.50	2.50	4.50	–	–
Acetone	1.25	2.50	0.65	1.25	–	–
Positive control						
Ampicillin	0.65	0.80	0.65	0.80	0.65	0.80

–, No activity. Data are given as activity index ± SEM.

P. appendiculatum (MIC = 0.65–1.25); moderately sensitive to ethanol extract of *M. polymorpha* and methanol extract of *D. undulatum* (MIC = 1.25–1.50), and less sensitive to the rest of the extracts (MIC = 2.50). MIC/MBC values clearly indicated that all the extracts were bacteriostatic, whereas ethanol extract of *M. polymorpha* was bactericidal. AI of *M. polymorpha* and *D. undulatum* in different organic solvents were found significantly different against *M. plutonius* at 5% level. However, in *P. appendiculatum* activity index of methanol and ethanol extracts were observed statistically non-significant (Table 1).

Although majority of the organic extracts showed varying levels of activity against bacterial pathogens, the acetone extracts of liverworts and chloroform extract of moss were found to be more active than other fractionated extracts. This can be explained due to the presence of specific antibacterial compounds in these extracts. This is consistent with the study of Dubey *et al.*[12] and Ilhan *et al.*[13], reporting a broad range of antibacterial effects of acetone extract of *Conocephalum conicum*, *M. polymorpha*, *P. appendiculatum* and *Pallustriella commutata* (Hedw.) Ochyra. The study is of considerable interest as all the organic extracts of the tested bryophytes were able to inhibit *M. plutonius*, a Gram-positive bacterium. The antibacterial activity of mosses and liverworts against Gram-negative bacteria has also been reported[13–15]. Bodade *et al.*[16] found that chloroform, acetone and ethanol extracts were more effective on *Escherichia coli* and *Staphylococcus aureus*. The variation in the susceptibility of the organism could be due to an intrinsic property related to the permeability of the cell surface to the extracts[17].

MIC of chloroform extract of *D. undulatum* was lowest (0.65). The MBC value for petroleum ether extract of *D. undulatum* (5.0) was higher than the others. Higher value of MBC is consistent with the study by Mewari and Kumar[18] and can be attributed to a crude form of bioactive compounds present in the extract. Even the positive control had bacteriostatic activity as evidenced by different values of MIC and MBC. These values were the same for ethanol extract of *M. polymorpha*, suggesting its bactericidal nature. Identical values of MIC and MBC can be explained due to the presence of specific bioactive compounds. Overall, antimicrobial activity might be due to the presence of flavonoids, steroids, terpenoids and other polyphenolic compounds[13] in the bryophytes. The results provide an effective and eco-friendly alternative to conventional antibiotics generally employed as preventive and curative agents for the control of the disease. However, further studies are necessary for isolation, purification and characterization of the active principles involved.

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An analysis of GPS-derived velocities in the Bengal basin and the neighbouring active deformation zones

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The Bengal basin, the largest fluvio-deltaic sedimentary system in the world, located in an area covering Bangladesh and three eastern states of India (West Bengal, Assam and Bihar) has been formed by sediments brought by the Ganga, Brahmaputra and Meghna rivers. This complex foreland basin originally emerged on a trailing margin of the Indian continental crust and was later complicated by convergence with Eurasia to the north and oblique convergence with Burma to the east. Apart from these tectonic events, another major source of crustal deformation in the vicinity of the Bengal basin was the formation of the Ninety East Ridge (NER) in the Indian Ocean. The Bengal basin, which is in the near vicinity of these three active boundaries, needs to be studied thoroughly for assessing seismic hazard in this region. A brief discussion of the tectonics of the neighbouring active zones is given here. The GPS-derived velocities of stations located in these zones and that at Kolkata, located in the Bengal basin show that the Kolkata–Coco Island baseline crossing the NER shortens at 18.5 ± 1.3 mm/yr, whereas the baseline between Kolkata and Aizawl, Mizoram shortens at 10.5 ± 1.5 mm/yr. The Kolkata–Siliguri baseline shortens at 8.1 ± 1.5 mm/yr and the Kolkata–Baradighi baseline shortens at 5.2 ± 1.4 mm/yr. The difference in shortening rates of these two stations located in the North Bengal foothill Himalayan zone relative to Kolkata is due to the presence of a highly active transverse zone lying between them.

Keywords: Baseline shortening, crustal deformation, GPS-derived velocity, seismic hazard.

ACCORDING to Mukhopadhyay and Krishna[1], the Ninety East Ridge (NER), which extends from 30°S northward into the Bay of Bengal, is buried beneath the Bengal Fan sediments. According to Curray *et al.*[2], NER is an aseismic ridge representing a hotspot trace. But according to Weins *et al.*[3], NER is a broad seismic zone considerably more active than the interior of any other oceanic plate. Seismic and gravity data and a study of earthquake focal mechanism suggest that NER is probably at the initial stages of subduction under the Andaman arc coupled with partial left lateral strike–slip motion along the ridge on its northern segment[4]. Wiens *et al.*[5] have proposed a diffused plate boundary (DPB), a zone of concentrated seismicity,

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