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Studies on influence of selected weeds on phosphorus solubilizing bacteria of Mizoram, northeast, India

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Abstract
A weed is commonly defined as a plant that grow out of place and is competitive, persistent and pernicious. The common dominant weeds of Mizoram that are used for the study of the antibacterial activity are: *Blumea lanceolaria*, *Spilanthes acmella*, *Bidens pilos* and *Chromolaena odorata*. Generally, these weed species are dominant during cultivation and the early successional phase after shifting cultivation in north eastern India. Phosphorus solubilizing bacteria (PSB) play an important role in phosphorus nutrition by enhancing its availability to plants through release from inorganic and organic soil P pools by solubilisation and mineralization. Bacillus species produce 167 biological compounds active against bacteria, fungi, protozoa and viruses. Among the four common weeds tested, *C. odorata* gave the largest area of inhibition zone against the two bacteria tested i.e., *B. subtilis* and *B. pumilus.* The study revealed that, the weed plants *C. odorata* may be cultivated for modern therapeutics to prepare potent antibacterial drugs.

Keywords: Weeds, phosphorus solubilizing bacteria, *Bacillus subtilis*, *Bacillus pumilus*, *Chromolaena odorata*

Introduction
A weed is commonly defined as a plant that grow out of place and is competitive, persistent and pernicious (James *et al*., 1991) [1]. Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes (Bhattarai and Shreshta, 2009) [2]. Plants are sources of very potent and powerful drugs with antibacterial properties (Chopra *et al*., 1992; Iyengar, 1985) [3, 4]. An antibacterial is a compound or substance that kills or slows down the growth of bacteria. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century (Zaika, 1975) [5]. The common dominant weeds of Mizoram that are used for the study of the antibacterial activity are: *Blumea lanceolaria*, *Spilanthes acmella*, *Bidens pilos* and *Chromolaena odorata*. Generally, these weed species are dominant during cultivation and the early successional phase after shifting cultivation in north-eastern India (Arunachalam, 2002) [6]. Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphate (Goldstein, 1987) [7]. A considerably higher concentration of phosphate solubilizing bacteria is commonly found in the rhizosphere in comparison with non-rhizosphere soil (Katznelson *et al*., 1962.; Raghu, 1966) [8, 9]. *Bacillus pumilus* is a gram-positive, aerobic, spore forming, rod Bacteria that occurs in soil, water, air, and decomposing plant tissue. It is often found on the developing root system of soybean plants but it does not harm the plants. *Bacillus subtilis*, known also as the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium (Madigan *et al*., 2005) [10]. B. subtilis is rod-shaped, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions. Unlike several other well-known species, B. subtilis has historically been classified as an obligate aerobe, though recent research has demonstrated that this is not strictly correct (Nakano MM *et al*.,1998) [11]. Bacillus species produce 167 biological compounds active against bacteria, fungi, protozoa and viruses (Cordovilla *et al*., 1993) [12]. The objective of this study was to determine capsaicin content of these high yielding chilli varieties to better guide farmers, consumers and pharmaceutical industries. The objective was to prepare extraction from selected weeds and to study the antibacterial activity of methanol extract of selected weeds against Phosphorous solubilizing bacteria (PSB).

Material and Methods

Plant Extraction: 1kg of each fresh leaves of *Blumea lanceolaria*, *Spilanthes acmella*, *Bidens pilosa and Chromolaena odorata* were collected from MZU campus, Tanhril. The collected plant materials were washed with distilled water and then washed with 70% ethanol and dried under sunlight. After completely dried the plant material were grinded into powder with a grinder under sterilized condition. 100g of each plant powder were taken and washed separately with 1L of petroleum ether in a clean glass container (a round bottom flask) and closed with aluminium foil. The solution was subsequently shaken and then filtered with Whatman filter paper No.1. The residue was again washed with 500ml of methanol, closed tight with aluminium foil, again shaken every 30mins for one whole day and filtered. The filtrate was centrifuged at 2000rpm for 10mins and the clear supernatant was collected. The extract solvent was kept in a wide open beaker and evaporated in an oven below 45 °C and kept overnight. The final extract should be dried completely and kept immediately in a refrigerator or 0°C until use. This is the crude extract which should be processed for the experiment.

Preparation of crude extract for antibacterial assay: The crude extract of different plants was weighed into 100mg, 75mg, 50mg and 25mg and kept separately in an eppendorf tube. Each of the extracts was dissolved in 0.5ml of dimethyl sulphoxide (DMSO). To these, 1ml distilled water was added to obtain 100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml concentrations.

Test microorganisms: *Bacillus subtilis* (ATCC 11774) and *Bacillus pumilus* (ATCC 14884) which are both phosphorus solubilizing bacteria (PSB) were used for test organisms. They were re-cultured by nutrient broth medium and then incubated at a B.O.D incubator at around 30°C for 24hrs. 1 ml of the suspended culture was then transferred to nutrient agar medium.

Media preparation

a) Nutrient broth medium: Peptone -1.25g, Beef extract -0.75g, Sodium chloride -2g, distilled water -250ml. b) Nutrient agar medium: Peptone -5g, Beef extract -3g, Sodium chloride -8g, Agar -15g, Distilled water -1000ml.

Evaluation of antibacterial activity

The agar well diffusion method as described by Esimone *et al*., (1998) [13] was adopted for the study. 15 ml of molten nutrient agar was seeded with 1.0 ml of standardized broth cultures of the bacteria by introducing the broth cultures into sterile Petri dishes, incorporating the molten agar rotating slowly to ensure uniform distribution of the microorganisms and then allowed to solidify on a flat surface. Four (4) holes were made in the plates (5.0 mm diameter) using a sterile cork borer and to that 3.5ml of different concentration of different extracts were transferred into the holes using a Pasteur's pipette. The plates were allowed to stand for one hour for pre-diffusion of the extract to occur and were incubated at 270C for 24 hrs at a B.O.D incubator. At the end of incubation, the plates were collected and zones of inhibition that developed were measured. The extracts were made into different concentrations *viz*., 100, 75, 50 and 25mg/ml and by using the agar diffusion method the zones of inhibition were recorded.

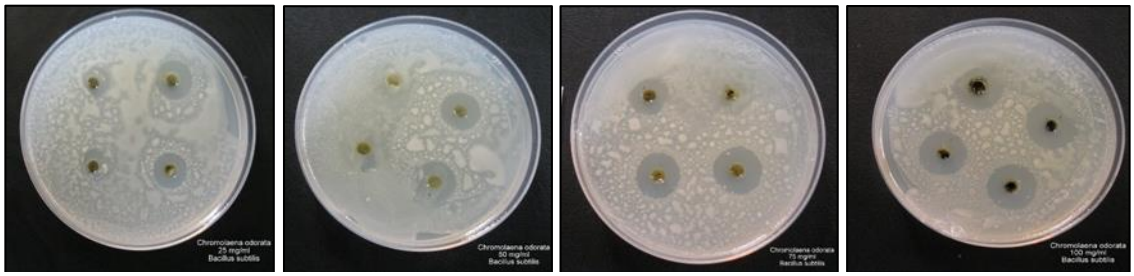
Results

***Blumea lanceolaria*:** Maximum inhibition zone was recorded at 100mg/ml and the minimum inhibition zone at 25 mg/ml in both the bacteria. The total mean of the extract on *B. subtilis* and *B. pumilus* was found to be 36mm and 41.25mm respectively; Table 1(a) and (b). The results show that the increase in concentration of the extracts increased the zones of growth inhibition of the bacteria i.e. 11, 9, 8.5 and 7.5mmon *B. subtilis* and 13, 12, 8.25 and 8mm on *B. pumilus* at the concentration of extracts of 100, 75, 50 and 25mg/ml respectively. The one-way analysis of variance (ANOVA), Table 2(a) and (b) showed significant variation ($P \leq 0.05$) of *B. subtilis* between 100 and 25 mg/ml. The significant variations of *B. pumilus* were also obtained between 100 and 25 mg/ml.

***Spilanthes acmella*:** The maximum zone of inhibition was recorded at 100mg/ml and the minimum inhibition at 25 mg/ml in both the bacteria i.e. 12.5, 12, 8.25 and 9mm on *B. subtilis* and 15.5, 13.5, 11.5, and 9mm on *B. pumilus* at the concentration of extracts of 100, 75, 50 and 25mg/ml respectively. The total mean of the extract on *B. subtilis* and *B. pumilus* was found to be 41.75mm and 49.5mm respectively; Table 1(a) and (b). The one-way analysis of variance (ANOVA) Table 2 (a) and (b), showed no significant variation ($P \leq 0.05$) of *B. subtilis* and *B. pumilus* in all the concentrations.

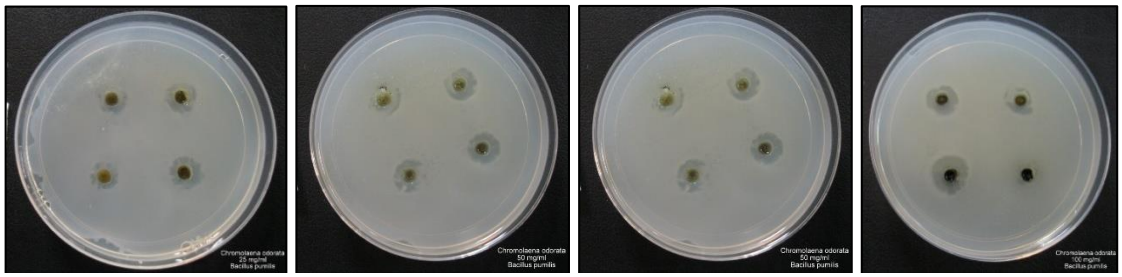
***Bidens pilosa*:** Maximum zone of inhibition was recorded at 100mg/ml and the minimum at 25mg/ml in both the bacteria i.e, 11, 9, 10 and 7.5mm on *B. subtilis* and 12, 11, 10 and 8.5mm on *B. pumilus* at the concentration of extracts of 100, 75, 50 and 25mg/ml respectively. The total mean of the extract on *B. subtilis* and *B. pumilus* was found to be 37.5mm and 41.5mm respectively; Table 1(a) and (b). The one-way analysis of variance (ANOVA), Table 2 (a) and (b), showed significant variation ($P \leq 0.05$) of *B. subtilis* between 100 75, 50 and 25, 100 and 50 and 100 and 25. The significant variation of *B. pumilus* were obtained between 100 75, 50 and 25, 100 and 25 mg/ml and 75 and 25mg/ml.

***Chromolaena odorata*:** Maximum zone of inhibition was also recorded at the highest concentration (100mg/ml) and minimum inhibition zone at 25mg/ml in both the bacteria i.e, 12.5, 12, 8 and 9mm on *B. subtilis* and 18, 16.5, 14.5 and 11.5mm on *B. pumilus* at the concentration of extracts of 100, 75, 50 and 25mg/ml respectively Figure 1 (a) and (b). The total mean of the extract on *B. subtilis* and *B. pumilus* was found to be 41.5 and 60.5 mm respectively; Table 1 (a) and (b). The one-way analysis of variance (ANOVA), Table 2(a) and (b), showed significant variation ($P \leq 0.05$) of *B. subtilis* between 100 and 25 mg/ml. Where *B. pumilus* showed no significant variation in all the concentrations.



25 mg/ml 50 mg/ml 75 mg/ml 100 mg/ml

Fig 1: (a): Effect of Chromolaena odorata against *B. subtilis.*



25 mg/ml 50 mg/ml 75 mg/ml 100 mg/ml

Fig 1: (b): Effect of Chromolaena odorata against *B. pumilus*

Table 1: (a): Zone of inhibition in *B. subtilis*

Concentration of extracts	Zones of inhibition (mm) of *B. subtilis*			
	B. lanceolaria	*S. acmella*	*B. pilosa*	*C. odorata*
100 mg/ml	11	12.5	11	12.5
75 mg/ml	9	12	9	12
50 mg/ml	8.5	8.25	10	8
25 mg/ml	7.5	9	7.5	9
Total	36	41.75	37.5	41.5

Table 1: (b): Zone of inhibition in *B. pumilus*

Concentration of extracts	Zones of inhibition (mm) of *B.pumilus*			
	B. lanceolaria	*S. acmella*	*B. pilosa*	*C. odorata*
100 mg/ml	13	15.5	12	18
75 mg/ml	12	13.5	11	16.5
50 mg/ml	8.25	11.5	10	14.5
25 mg/ml	8	9	8.5	11.5
Total	41.25	49.5	41.5	60.5

Table 2: (a): One-way analysis of variance (ANOVA) of *Bacillus subtilis.*

Sl.no.	Parameter	Source of variation	F-Ratio	P-value
1.	*Blumea lanceolaria*	100x75x50x25	3.46667	0.05089
		100x75	3.00000	0.13397
		100x50	3.94737	0.09413
		100x25	13.3636*	0.01063*
		75x50	0.15789	0.70485
		75x25	2.45455	0.16823
		50x25	0.85714	0.39026
2.	*Spilanthes acmella*	100x75x50x25	1.955	0.175
		100x75	0.055	0.823
		100x50	1.923	0.215
		100x25	3.419	0.114
		75x50	2.000	0.207
		75x25	3.857	0.097
		50x25	1.000	0.356
3.	*Bidens pilosa*	100x75x50x25	5.000*	0.018*
		100x75	3.000	0.134
		100x50	9.000*	0.024*
		100x25	13.36*	0.011*
		75x50	1.000	0.356
		75x25	2.455	0.168
		50x25	0.429	0.537
4.	*Chromolaena odorata*	100x75x50x25	2.857	0.082
		100x75	0.097	0.766
		100x50	3.429	0.114
		100x25	7.737*	0.032*
		75x50	1.174	0.32
		75x25	3.857	0.097
		50x25	2.455	0.168

Table 2: (b): One-way analysis of variance (ANOVA) of *Bacillus pumilus*.

Sl.no.	Parameter	Source of variation	f-ratio	p-value
1.	*Blumea lanceolaria*	100x75x50x25	1.888	0.186
		100x75	0.176	0.689
		100x50	2.445	0.169
		100x25	8.333*	0.028*
		75x50	1.291	0.299
		75x25	3.429	0.114
		50x25	0.008	0.933
2.	*Spilanthes acmella*	100x75x50x25	0.925	0.458
		100x75	0.189	0.679
		100x50	0.756	0.418
		100x25	3.271	0.121
		75x50	0.195	0.674
		75x25	1.653	0.246
		50x25	0.51	0.502
3.	*Bidens pilosa*	100x75x50x25	3.963*	0.036*
		100x75	0.600	0.468
		100x50	3.000	0.134
		100x25	9.800*	0.020*
		75x50	1.000	0.356
		75x25	6.818*	0.040*
		50x25	3.857	0.097
4.	*Chromolaena odorata*	100x75x50x25	2.297	0.13
		100x75	0.284	0.613
		100x50	1.861	0.221
		100x25	5.337	0.06
		75x50	0.686	0.439
		75x25	3.488	0.111
		50x25	1.543	0.261

Discussion

The study indicated that the methanolic extract of the plants inhibited the growth of the two Phosphorous solubilizing bacteria. This therefore, showed that the extracts contained substances that can inhibit the growth of the selected bacteria. In *Chromolaena odorata*, the plant extract had higher activity against *B. pumilus* among all the species of different concentrations with total inhibition zone of18mm. The highest inhibition zone for *B. subtilis* was 12.5mm at 100mg/ml and 18mm at 100mg/ml for *B. pumilus.* The chemical composition of *Chromolaena odorata* included terpenes, sesquiterpenes, triterpenoides, steroids, flavonoides, phenyl propaniods and their derivatives as well (Ye Min *et al*., 2008) [14]. Statistical analysis revealed that there was significant variation between most of the concentrations of the plant extract, except for *Spilanthes acmella* on both *B. subtilis* and *B. pumilus* no significant variation was obtained. So, it may be due to the presence of these chemical compounds and substances that the plant extracts can inhibit the growth of the bacteria. Thus, under different circumstances the weed plant itself may be cultivated for modern therapeutics to prepare potent antibacterial drugs.

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