Table 1. Synergistic ovipositional deterrence activity of extracts of *Glycosmis pentaphyllum* and other plants.

Compound	Dose (mg cm^{-2}) 5	2	1
G. pentaphyllum	20	14	10
Neemrich I	20	18	10
G. pentaphyllum + Neemrich I	35	30	15
S. oledodes	20	16	12
G. pentaphyllum + *S. oledodes*	45	38	30
C. roseus	5	3	2
G. pentaphyllum + *C. roseus*	10	6	2
Breneya sp.	10	4	2
G. pentaphyllum + *Breneya* sp.	15	5	2

*Day's persistence of 100% action.

agricultural and public health importance. Extracts of different plants such as *Catharanthus roseus*, Neemrich I[5], *Salvadora oleododes and Breneya* species were combined with *G. pentaphyllum* extract to study synergistic effects on OD activity against the potato tuber moth (PTM). Procedures standardized earlier[6] were used to determine persistence and nature of OD activity against PTM.

OD activity against the PTM adults was measured by length of persistence of 100% oviposition inhibition on a preferred substrate treated with desired doses of the plant extracts. The latter were used singly and in various combinations (Table 1). *G. pentaphyllum* gave maximum 100% OD activity at 5 mg cm^{-2} dose which persisted for 20 days. At lower doses (2.5 mg cm^{-2} and 1 mg cm^{-2}) the persistence level decreased. The same activity was also obtained in Neemrich I and *S. oleododes*. *C. roseus* and *Breneya* sp. did not exhibit any significant activity at 5 mg cm^{-2}. Combination of plant extracts, viz. *G. pentaphyllum* with Neemrich I, *S. oleododes*, *C. roseus*, and *Breneya* sp. in simple 1 : 1 ratio yielded significant increase in OD activity (Table 1). The combination *G. pentaphyllum* + Neemrich I showed double the persistence obtained with *G. pentaphyllum* alone at both high and low doses. Enhancement of OD activity was also obtained with combinations of *G. pentaphyllum* and *S. oleododes* at all doses. However, combinations of *G. pentaphyllum* with *C. roseus* and *G. pentaphyllum* with *Breneya* sp. exhibited only marginal increase in activity at all doses.

From the foregoing, it is apparent that combination of some plant extracts in appropriate proportions induces synergistic enchancement of OD activity at even half or lesser dose. This phenomenon can have important implications in the practical application of natural products for pest management.

1. Klocke James, A., *Allelochemicals—Role in Agricultural and Forestry* (ed. R. George Waller), American Chemical Soc., Washington 1985, p. 396.
2. Sharma, R. N., Workshop on Futurology on use of Chemicals in Agriculture with Particular Reference to Future Trends in Pest Control, Coimbatore 1979, p. 17.
3. Barton, L., *Browne Chemical Control of Insect Behaviour* (eds. H. H. Shorey and J. J. Mckelvey), Wiley Interscience Publications, New York, London, Sydney, Toronto 1977, p. 117.
4. Deshpande, S. G., Sharma, R. N. and Nagasampagi, B. A., Proceedings of National Symposium on Integrated Pest Control Progress and Perspectives, 1988, p. 392.
5. Sharma, R. N., Nagasampagi, B. A., Bhosale, A. S., Kulkarni, M. M. and Tungikar, V. B.. Proceedings of 2nd International Conference Ravischholzhausen, 1983, p. 115.
6. Sharma, R. N. *et al.*, *Z. Naturforsch.*, 1981, **C112**.

1 February 1990

Occurrence of phosphate-solubilizing bacteria in the endorhizosphere of crop plants

P. U. Krishnaraj and T. K. Siddarame Gowda*
Department of Agricultural Microbiology. University of Agricultural Sciences, Dharwad 580 005. India
*Department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore 560 065, India

Plant roots examined harboured phosphate-solubilizing bacteria in the endorhizosphere, with *Beta vulgaris* harbouring the highest population.

ENDORHIZOSPHERE refers to the root interior, including the epidermis–cortex region[1]. The presence of bacteria in this region capable of fixing atmospheric nitrogen and production of growth regulators is well known. It is possible that these roots also harbour other types of beneficial bacteria. Hence the present study was conducted to explore the possibility of the presence of phosphate-solubilizing bacteria in the endorhizosphere of different crops.

The method followed was essentially the same as described by Watanabe *et al.*[2] Ten grams of the root sample was transferred into a 250 ml conical flask containing 100 ml of sterile distilled water and 5 g of glass beads. The roots were washed by shaking for 5 min. The washed water was decanted and fresh 100 ml sterile water was added. The washings were repeated ten times to remove all the soil particles and micro-organisms present on the root surface. The washed roots were transferred under aseptic conditions to a mortar which was previously sterilized with 70% alcohol, flamed and cooled. The roots were cut aseptically into small pieces (2–3 cm), macerated and serially diluted. The appropriate dilution was plated on Tryptic soy agar medium (Difco) and hydroxyapatite agar medium[3] to enumerate the total bacterial and total phosphate-solubilizing bacterial population respectively.

Table 1. Population of bacteria in endorhizosphere of different crops.

Crop	Bacterial population on TSA ($\times 10^6$/g root)	Phosphate-solubilizing bacteria ($\times 10^6$/g root)
Mango	37.92 ± 3.75	3.67 ± 0.25
Carrot	28.78 ± 5.01	7.25 ± 1.0
Maize	24.11 ± 4.01	7.05 ± 0.37
Beet root	22.50 ± 4.17	10.58 ± 0.75
Ragi	13.22 ± 1.79	4.15 ± 0.29
Sapota	10.36 ± 1.07	1.93 ± 0.14
Mulberry	7.15 ± 0.72	2.74 ± 0.6
Tomato	3.85 ± 0.77	0.23 ± 0.8
Cashew	2.50 ± 0.83	0.21 ± 0.4

± Indicates S.E.

Table 1 indicates that all plant roots harboured bacteria in the endorhizosphere to varying degrees. The highest population was observed in mango (3.79×10^7/g root) and the least in cashew (2.5×10^6). The present study also reveals that the phosphate-solubilizing bacteria are present in the endorhizosphere of the crop plants. They range from about 4% to 84% of the total endorhizosphere bacteria. Beet (*Beta vulgarts*), a non-mycorrhizal plant, harboured the highest number of phosphate-solubilizing bacteria. It is possible, therefore, that non-mycorrhizal plants may have higher population of phosphate solubilizers than mycorrhizal plants. Considerable work has already been done for studying the occurrence, distribution and ability of the bacterial isolates associated with crop plants particularly in the rhizosphere and soils[4]. However, to our knowledge no information is available on the presence of phosphate-solubilizing bacteria associated with endorhizosphere of any plant. Therefore, the information gathered in this study possibly opens a new area of research to understand the role of phosphate-solubilizing bacteria in the phosphorous nutrition of crop plants, because unlike rhizosphere and soil micro-organisms, the endorhizosphere micro-organisms are closely associated within the plants with greater degree of specificity.

1. Balandreau, J. and Knowles, R., *Interaction Between Non-pathogenic Soil Micro-organisms and Plants*, Elsevier/North Holland Publishing Co., Amsterdam, 1978, p. 243.
2. Watanabe, I., Barraquio, W. L., De Guzman, M. E. and Cabrera, B. A., *Appl. Environ. Microbiol.*, 1979, **37**, 813.
3. Sperber, J. I., *Aust. J. Agric. Res.*, 1958, **9**, 778.
4. Cosgrove, U. T., *Adv. Microbiol. Ecol.*, 1977, **1**, 95.

9 January 1990

A simple method for clonal culture of *Entamoeba histolytica*

Manjula Srivastva, C. M. Habibullah and S. R. Das*
Department of Gastroenterology, Osmania General Hospital, Hyderabad 500 012, India
*Division of Microbiology, Central Drug Research Institute, Lucknow 226 001, India

A simple and reliable method was developed for clonal culture of *Entamoeba histolytica*. Strains of *E. histolytica* were isolated from different categories of amoebiasis cases and were maintained in Boeck and Drbohlav (B&D) medium. They were cloned in an overlay of B&D medium. Ten amoebae each were cloned from acute amoebic dysentery (AAD) and non-dysenteric amoebic colitis (NDAC) cases and six were cloned from amoebic liver abscess (ALA) cases. The success rate was 80, 60 and 83% for AAD, NDAC and ALA cases respectively. This method gives a better success rate than other methods of establishment of clones from different categories.

ANY culture of *Entamoeba histolytica* established *in vitro* will consists of heterogenous population of amoebae, i.e. they contain both pathogenic and non-pathogenic species. Even a single species may contain heterogenous population, e.g. NIH:200. The variation may be due to many factors like mutations, toxic substances secreted by amoebae and chemicals present in the medium. Therefore, the clones from indigenous strains of *E. histolytica* are desirable for immunological, biochemical, and genetic studies.

Reports are available for the clone cultures of *E. histolytica*[1-6]. Most of these workers cloned *E. histolytica* either from axenic cultures or with other species of Entamoebae. As far as we know only Farri[3] has isolated and cloned *E. histolytica* from different categories of amoebiasis. In the present report an attempt was made to initiate and maintain clone cultures of *E. histolytica* in Boeck and Drbohlav (B&D) and in liquid media.

B&D medium was prepared according to the method of Boeck and Drbohlave[7]. Liquid medium for clone cultures was prepared as follows: An overlay of B&D medium was pooled out in a screw-cap flat bottom flask after 24 h of incubation. It was mixed with inactivated bovine-serum in the ratio of 9:1 (9 parts pooled overlay + 1 part bovine serum).

The faecal samples positive for *E. histolytica* were incubated in B&D medium. These samples were taken from acute amoebic dysentry (AAD) and non-dysentric amoebic colitis (NDAC) cases and were subcultured regularly after every 48 h.

The clones were initiated from xenic cultures of B&D medium. A drop of profuse growth of xenic cultures was taken on a microscopic slide and was diluted with