negligible activity.

To test the effect of the bacterium on anthracnose disease intact jute plants were first sprayed with either dilute suspension of the bacterium or its cell-free culture filtrate. After 24 hr, selected plants were inoculated with the spore suspension of *C. corchori* ($10 \times 10^6$ spores/ml) and incubated for 72 hr for lesion formation. Besides, in order to substantiate the results of this experiment, some treated (either with bacterial suspension or culture filtrate) leaves were detached, inoculated with *C. corchori* and incubated under moist conditions at room temperature (30–32° C). The results of both the experiments are given in table 1.

TABLE 1

*In vivo effect of the bacterial suspension and the cell free culture filtrate of B. megaterium on lesion production by C. corchori.*

| Inoculation | Treatment | *% inhibition of lesion production | *% reduction in lesion spread |
|---|---|---|---|
| On detached leaves** | Bacterial suspension | 88 | 95 |
| | Culture filtrate | 75 | 44 |
| On intact plants # | Bacterial suspension | 57 | 82 |
| | Culture filtrate | 21 | 16 |

*In relation to distilled water controls.
**Incubated for 48 hr and 96 hr.
# Incubated for 72 hr and 120 hr.

Thus, *Bacillus megaterium* (B-23) could be used quite effectively for controlling anthracnose disease of jute caused by *C. corchori* (figure 1). The bacterial suspension appears to be more effective than its cell-free culture filtrate probably because the bacteria can multiply rapidly on the leaf surface before inoculation with the test organism. The antagonistic effect is not due to competition for nutrients alone since the culture filtrate can also reduce disease incidence on the leaf surface to a significant extent. In a similar attempt to control anthracnose of cucumber seedlings, Leben and Daft[9] have used washed cells of *Pseudomonas* sp. A-180. The bacterium was sprayed 24 hr prior to inoculation with the test fungus which effectively controlled the disease. Swinburne[10,11] also succeeded in controlling leaf scar of apple (caused by *Nectria galligena*) by prior inoculation with the bacterial suspension of *Bacillus subtilis*.

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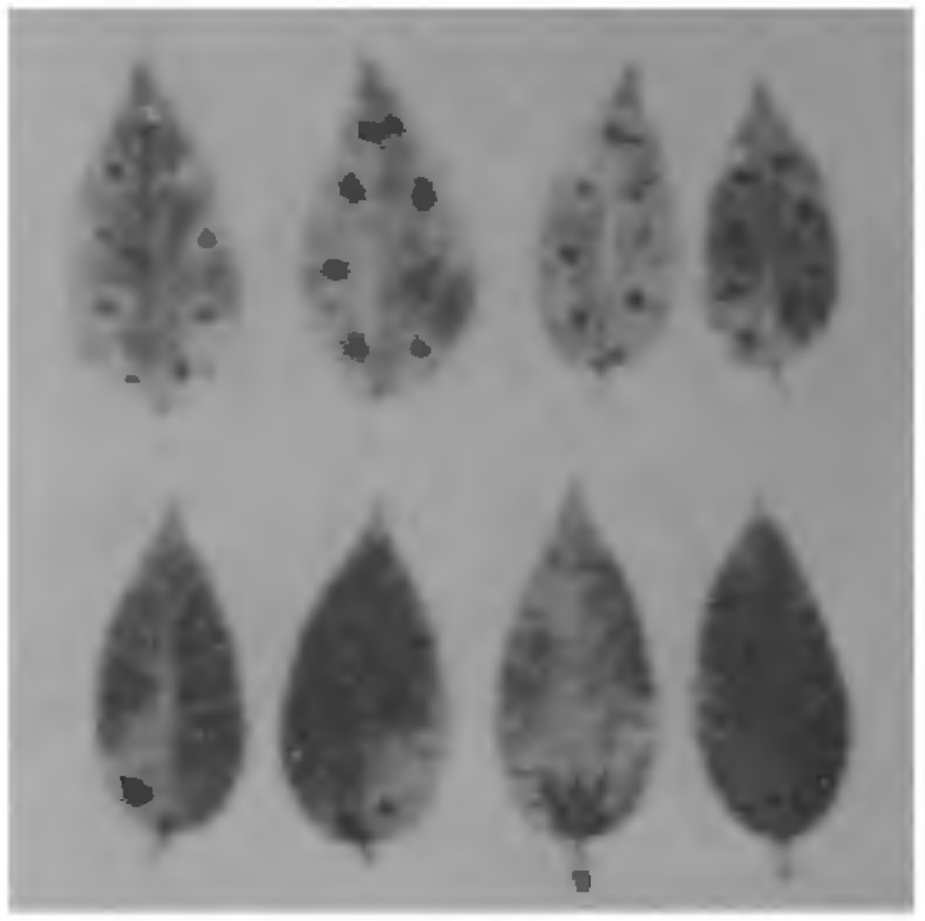

**Figure 1.** Upper leaves with lesions caused by *C.corchori*, lower leaves treated with bacterial suspension showing a few small lesions.

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## SURVIVAL OF *RHIZOBIUM JAPONICUM* IN CHARCOAL BENTONITE BASED CARRIER

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A MAJOR breakthrough in legume inoculant

production in India came in 1969 when Indian peat soil was considered suitable as a carrier for commercial production of legume inoculants[1]. But scarcity of quality peat in India soon diverted the attention of *Rhizobium* workers towards the need to evaluate the utility of several other indigenously available substrates as carriers for *Rhizobium*. Among many substrates used as carrier for *Rhizobium*, charcoal found favour probably due to its capacity for adsorption of toxic compounds and aeration of the medium conducive for enhancing the longevity of the bacteria[2,3]. Charcoal based legume inoculants are quite popular in India. In view of improving the quality of charcoal based inoculants in the present study, the charcoal was amended with bentonite and its effect was studied on the moisture content of the carrier and the shelf-life of the inoculant. The results of the study are reported here.

Peat and charcoal + soil (75 : 25) mixture were sampled through 100 mesh sieve and neutralised with finely powdered calcium carbonate. The samples were used with and without $K_2HPO_4$(0·5%). Samples of charcoal + soil (75 : 25) mixture containing 0·5% $K_2HPO_4$ were amended with bentonite at the rate of 5 and 10%. The treatments are listed in tables 1 and 2. Samples were steam-sterilised at 15 psi pressure (121°C) for 4 hr. An efficient strain of *Rhizobium japonicum* (SB-16) was grown in yeast-extract-mannitol broth medium by shaking it continuously for 7 days at 28-30°C on a rotary shaker. The broth culture of *Rhizobium japonicum* (having a titre value $106 \times 10^8$ cell/ ml) was added to the carrier

TABLE 1

*Survival of Rhizobium japonicum in various carrier at 35°C. (Average of 3 replicates)*

| Carrier | Log No. of viable cells/g of carrier (weeks) | | | | | |
|---|---|---|---|---|---|---|
| | 2 | 4 | 8 | 12 | 16 | Mean |
| Peat | 9·0979 | 9·0000 | 8·7966 | 8·3680 | 7·9912 | 8·6507 |
| Peat + $K_2HPO_4$ | 9·1226 | 9·0086 | 8·8692 | 8·6163 | 8·0141 | 8·7262 |
| Charcoal + soil | 8·8770 | 8·7505 | 8·4362 | 7·9009 | 7·2553 | 8·0439 |
| Charcoal + soil + $K_2HPO_4$ | 8·9912 | 8·8531 | 8·6596 | 8·2923 | 7·6335 | 8·4859 |
| Mean | 9·0222 | 8·9031 | 8·6904 | 8·2944 | 7·7235 | |

C.D. at 5% (treatment) =0·2031
C.D. at 5% (period) =0·1759

TABLE 2

*Survival of Rhizobium japonicum in charcoal + soil (75 : 25) amended with bentonite at 35°C. (Average of 3 replicates)*

| Carrier | Log No. of viable cells/g of carrier (weeks) | | | | | |
|---|---|---|---|---|---|---|
| | 2 | 4 | 8 | 12 | 16 | Mean |
| Charcoal + soil + $K_2HPO_4$ (control) | 8·6684 (1·3766) | 8·6128 (1·3243) | 8·4150 (1·2201) | 8·0000 (1·0645) | 7·6021 (0·8062) | 8·2597 (1·1583) |
| Charcoal + soil + 5% bentonite + $K_2HPO_4$ | 8·6749 (1·4472) | 8·6160 (1·4065 | 8·3802 (1·3139) | 8·0792 (1·1931) | 7·7160 (1·0128) | 8·2933 (1·2747) |
| Charcoal + soil + 10% bentonite + $K_2HPO_4$ | 8·6812 (1·5132) | 8·6464 (1·4579) | 8·5441 (1·3838) | 8·2553 (1·2923) | 8·0607 1·1523) | 8·4375 (1·3599) |
| Mean | 8·6748 (1·4457) | 8·6251 (1·3962) | 8·4464 (1·3059) | 8·1115 (1·1833) | 7·7929 (0·9904) | |

C.D. (treatment) at 5% 0·1405 (0·0657)
C.D. (period) at 5% 0·1816 (0·0848)

figures in parenthesis are log of moisture contents (%) in carrier at various intervals.

samples and moisture contents were brought to 40% of their water holding capacity, inclusive of the water carried by the inoculum. after inoculation, the material was packed in polythene bags @ 200 g/ packet in triplicate and the packets were incubated at 35° C. The samples were analysed for bacterial counts at regular intervals by plate method in the yeast-extract-mannitol-agar medium containing congored (1:400)[4]. The moisture contents of the carrier samples were also determined at regular intervals.

Data on survival of *R. japonicum* in various carrier mixtures (table 1) revealed that the addition of $K_2HPO_4$ (@ 0·5%) to charcoal proved advantageous and significantly enhanced the shelf-life of inoculant. It is also interesting to observe that the addition of bentonite @ 10% to the carrier samples helps in maintaining significantly higher moisture contents and population of *Rhizobium* in the carrier for longer duration (table 2). This confirms the findings of Strijdom and Deschodt[4].

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## OCCURRENCE OF $A^2$ MATING TYPE OF BETELVINE *PHYTOPHTHORA* IN JABALPUR, M.P., INDIA

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It is now established that many of the heterthallic species of *Phytophthora* are potentially homothallic but require some stimulatory substance(s) for development of sex organs and oospore formation[1-3]. Oospore formation in one such species, *Phytophthora nicotianae* var. *parasitica* (Dastur) Waterhouse (=*Phytophthora parasitica* var. *piperina* Dastur), the causal organism of foot rot and leaf rot of *Piper betle* L. ('Pan'), though earlier reported[4], has been demonstrated under laboratory condition as well as in nature in a recent study undertaken at Sagar, M.P.[5] However, no evidence about the occurrence of two mating types of the fungus which are required for oospore formation or involvement of stimulatory substances has been given.

While studying the leaf surface microflora of *P. betle* in relation to *Phytophthora* leaf rot disease, a search for mating types of the pathogen isolated from different betelvine growing areas in Jabalpur division was also made which is reported in the present note.

The infected 'Pan' leaves from betelvine orchards at Gupteshwar (Jabalpur), Mandla, Narsinghpur, Sihora and Katni were collected during the rainy season of 1979 and isolations of the pathogen were made in potato dextrose agar medium by usual procedures. Fifteen single zoospore isolates, three from each area, were at first paired among themselves in all possible combinations by keeping 4 mm mycelial discs about 4½ mm apart in Petri plates containing oatmeal agar medium unamended and amended with 0·1 g/l calcium chloride and 200 mg/l cholesterol. Besides, all isolates were grown alone on unamended and amended oatmeal agar media. Mating types of these isolates were further determined by pairing $A^1$ (P 991) and $A^2$ (P 731) mating types of *P. parasitica* Datsur, provided through the courtesy of Dr. W. H. Ko on unamended and amended oatmeal agar. The plates were incubated at 25° C in darkness for 8–10 days after which mycelial strips from the point of contact between the colonies of paired isolates were picked up. These were boiled in distilled water for 10 min to remove the adhering medium, stained in 1% aqueous solution of cotton blue and mounted in lactophenol for observation of oospore formation.

All the fifteen isolates failed to form oospore in monoculture and also when paired among themselves on unamended and amended media. However, production of sporangia was greatly enhanced in the amended medium as compared to the unamended one. When paired with $A^1$ and $A^2$ mating types of *P. parasitica*, oospore with persistent amphigynous antheridia were formed in all the isolates in case of pairing with $A^1$ mating type only. Amended oatmeal agar medium was found slightly superior to unamended one for oospore formation also.

These results indicate that betelvine *Phytophthora*, common in different 'Pan' growing areas of Jabalpur division is not homothallic; even substances like cholesterol and calcium chloride do not stimulate the oospore formation. All the isolates of pathogen therefore are heterothallic and belong to $A^2$ mating type, requiring $A^1$ mating type for oospore formation.

Our grateful thanks are due to Dr. W. H. Ko, Department of Plant Pathology, University of Hawaii, Hawaii, U.S.A. for kindly providing sub-cultures of $A^1$ and $A^2$ mating types of *P. parasitica* and to the Principal, Govt. Science College, Jabalpur for facilities. One of us (A.K.A.) is grateful to I.C.A.R., New Delhi for the award of a fellowship.

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