

were not apparently infected by the parasitic fungus. The sporodochial development was also observed on adults which subsequently died. The dead crawlers and adults were found adhering on the plant surface by a mucilaginous substance probably secreted by the parasite.

The perithecia developed on dead crawlers and adults which were earlier killed by sporodochial development. Perithecial formation invariably followed sporodochial development and was not observed to develop independently.

The sporodochia were creamy white to orange coloured, 600–900  $\mu$  in diameter and contained subhyaline angular cells. Conidiophores were produced densely on the surface of sporodochial stroma, moniliform, subhyaline and measured 17.5–28  $\times$  3–8  $\mu$ . Conidia were typically staurosporous, hyaline, 3-armed, fusiform with acute apex, 10–20 septate, arms united at the base and measured 90–175  $\times$  5.5–7  $\mu$ .

Perithecia were solitary to gregarious, globose to subglobose, creamy white to dull brown, mostly smooth (rarely hairy), walls composed of globose cells and measured 350–580  $\mu$  in diameter. Numerous filiform, hyaline pseudoparaphyses were intermixed with asci. Asci typically bitunicate, subhyaline, cylindrical-clavate and measured 150–245  $\times$  15–27  $\mu$ . Ascospores octosporous, long, vermiform, hyaline, 10–20 septate with rounded apex and narrowly rounded base and measured 85–200  $\times$  6.5–8  $\mu$ .

Taxonomical characters of the parasitic fungus indicated its identity in the perithecial stage to *Podonectria coccicola* (Ellis and Everh.) Petch and in its conidial stage to *Tetracrium coccicolum* Höhn, to which they are referred respectively. Occurrence of the fungus and its parasitism on scale insects hitherto been unreported from this country, constituting first record for India.

The genus *Podonectria*, established by Petch<sup>3</sup>, includes species parasitic on scale insects. Some of these are: *Podonectria coccicola*, *P. aurantii* (Höhn) Petch, *P. echinata* Petch<sup>3</sup>; *P. nova-Zealandica* Dingley, *P. gahnia* Dingley; *P. tenuispora* Dennis<sup>1</sup> and *P. coccorum* (Petch) Rossman<sup>2</sup>. *Podonectria coccicola* is considered as important in the biological control of destructive scale insects on citrus trees<sup>6</sup>. Rolfs and Fawcett<sup>4</sup> gave a method for spreading these fungi in citrus orchards to control scale insects. At present *P. coccicola* has become extinct in the United States although at one time it was an important biological control agent (Rossman<sup>6</sup>, personal communication). This species has been observed to occur abundantly in Coorg area of Karnataka State, where high rainfall and moderate temperatures prevail. In most of the cases there was a high rate of natural infection and heavy mortality of scales. The parasitic fungus can

be exploited as an important biological control agent of scale insects.

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#### A SIMPLE TECHNIQUE FOR ISOLATING *PHYTOPHTHORA PALMIVORA* FROM SOIL

DESPITE much work, the methods of detection and isolation of *Phytophthora palmivora* (Butl.) Butl. from soil are meagre. Baiting with plant materials has been the most commonly used method for isolating fungi from soil<sup>4</sup>. Fatemi<sup>2</sup> used hemp and sawflower seeds as baits for isolating different species of *Phytophthora* and *Phthium* from soil; cucumber seeds were also tried with success for the same fungi<sup>1</sup>. Lupin and apple baits are in use for isolating *P. cinnamomi*<sup>3</sup>. Zentmyer *et al.*<sup>6</sup> found Fuerto fruits to be excellent traps for isolating *P. cinnamomi*. Many attempts to isolate *P. palmivora* from soil by using different baits such as apple, lemon, cucumber, carrot, tomato and brinjal proved unsuccessful. When freshly exised root bits of *Colocasia esculenta* were used as baits, following the method of Subramanian<sup>5</sup>, *P. palmivora* was found to colonise the root bits readily. The method is described here.

Root bits of 1" length were cut from mature *Colocasia* plants and were washed under running tap water to remove the adhering soil. The root bits were autoclaved at 10 lb pressure for 15 minutes. The sterile root bits were then transferred to Petri plates (5 bits per plate), each containing 50 g of soil



collected from *Phytophthora*-infested *Colocasia* fields in Hyderabad. Sufficient sterile water was added to keep the soil moist. The plates were incubated for one week at  $15^{\circ}\text{C} \pm 2^{\circ}\text{C}$  in a cold room. After the incubation the root bits were removed and washed thoroughly with sterile water for several times and blotted dry. Then the root bits were plated on oat meal agar medium aseptically. After 4 days white fluffy growth of *P. palmivora* was observed and the fungus was transferred to oat meal agar slants.

The present root baiting technique has given promising and more reproducible results than any other baits tried and would be useful in survival studies of *P. palmivora* in soils.

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#### PRODUCTION OF PROTEASE BY *SCLEROTINIA SCLEROTIORUM* CAUSE OF WHITE ROT OF *BRASSICA JUNCEA*

WHITE ROT caused by *Sclerotinia sclerotiorum* is a very destructive disease and causes heavy economic losses to *Brassica juncea* crop in north-eastern India<sup>1</sup>. During the course of the physiopathological studies, certain aspects of the pathogenesis were investigated and the present paper deals with the production of enzyme protease in susceptible and resistant cultivars at different stages of pathogenesis.

##### Experimental

Four isolates belonging to 4 different groups, viz., "Most highly", "Highly", "Moderately" and "Weakly" virulent isolates and cultivars of *B. juncea* showing maximum and minimum disease reactions against these pathogens. Preflowering plants of both the varieties were inoculated by agar disc method of Rai and Dhawan<sup>2</sup>. Infected plant parts were harvested after 5, 10 and 15 days of inoculation. Samples were homogenized with 0.1 M phosphate

buffer (pH 7.0) in a ratio of 1 : 5 (w/v), strained and centrifuged at 6000 rpm for 15 minutes, supernatants were used as crude enzyme preparation.

**Enzyme assay :** Proteolytic activity was determined by the modified method of Davis and Smith<sup>3</sup>. Reaction mixture consisted of 2 volumes of 1% casein in 0.1 M phosphate buffer (pH 7), 1 volume of the same buffer and 1 volume of the enzyme preparation was incubated at  $30^{\circ}\text{C}$ . Aliquots of 1.0 ml of reaction mixture were withdrawn after 2 hrs and immediately treated with 2 ml of ninhydrin reagent which stopped the reaction. Tubes were kept in boiling water bath for 20 minutes to develop the colour and were cooled to room temperature. Final volume was made up to 50 ml with 50% *n*-propanol and the colour intensity measured at 570 m $\mu$ . The enzyme activity was expressed as nanomoles of  $\alpha$ -amino nitrogen released at  $30^{\circ}\text{C}$  per ml of reaction mixture in the specified time of reaction.

##### Results and Discussion

During this study all the isolates of *S. sclerotiorum* were found to produce significant amount of protease in both the host varieties (Table I). It was more or

TABLE I

*Protease activity\* (nanomoles of aminonitrogen released/ml of reaction mixture/2 hr) in the tissue extract of susceptible and resistant B. juncea plants after 5, 10 and 15 days after inoculation with 4 different isolates of S. sclerotiorum*

Isolates of <i>S. sclerotiorum</i>	Days after inoculation	Susceptible host tissue	Resistant host tissue
Ss-1 (MHV)	5	1201.97	1156.62
	10	1220.81	1173.62
	15	1245.91	1154.88
Ss-2 (WE)	5	1151.73	1082.73
	10	1161.11	1117.25
	15	1201.97	1054.88
Ss-3 (HV)	5	1085.86	1011.20
	10	1114.10	1098.41
	15	1139.21	950.91
Ss-4 (MV)	5	1180.01	1121.95
	10	1189.43	1132.93
	15	1220.71	1062.22

MHV, Most highly virulent; MV, moderately virulent; HV, highly virulent; WE, weakly virulent.

\* Data presented in the table are average of 3 independent assays.

Note : Control sets showed no activity.