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Cell Wall Degrading Enzymes Production by Seed-Borne Fungi Isolated from Chilgoza (*Pinus gerardiana* Wall)

Pushp Lata Singh*, M. N. Gupta* and A. L. Singh

National Research Centre for Groundnut, Timbawadi, Junagadh-362 001

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The chilgoza (*P. gerardiana* Wall) seed because of its high oil, fat and protein is severely, attacked by fungi and losses viability, if stored for one year in ambient atmosphere (Singh *et al*., 1988). The present investigation was carried out to study the production of cell wall degrading enzymes produced by fungi actively associated with fresh and stored seed of chilgoza.

Nine fungi as listed in the Table 1 isolated from chilgoza seed (Singh, 1982) were used in this study. The medium containing 1.0 g Pectin (Citrus), 0.5 g glucose, 0.1 g KH_2PO_4, 0.05 g $MgSO_4$, 0.213 g ammonium nitrate and 100 ml sterilized distilled water was used to test the production of enzymes. The liquid medium 12 ml was taken in flat bottles and autocloved at 15 lbs psi for 20 mts. The fungus was inoculated taking four small bits of one mm diameter from 7 days old culture. The bottles were shaken carefully to spread the inoculum and stacked in an incubator at 25±1°C. After 5 days the mycelium met was filtered through whatman No. 1 filter paper. The filtrate was centrifuged for 20 minutes at 3000 r.p.m and supernatant was used for estimating the protopectinase (PP) activity by potato disc method, pectinesterase (PE) acting by titration method, and polygalacturonase (PG) activity by method as described by Khare *et al*. (1979). For C_1 cellulase enzyme 2% each of agar and cellulose powder was used and the enzyme extract was prepared after 7 and 15 days of incubation. The C_x cellulase activity was measured by viscocity method (Hussain and Rich, 1958) and C_1 celluase activity by column clearing technique of Rautella and Cowling (1966).

For testing *in vivo* production of enzymes by these fungi, 20g fresh and one year stored of chilgoza seeds were surface sterilized with 0.1% $HgCl_2$ solution and soaked in sterilized distilled water in a small beaker. After 16 h of

* Botany Department, Agra College Agra, U.P., India