Hyphal interaction between *Trichoderma koningii* and *Ustilago segetum tritici* through scanning electron microscopy

Trichoderma koningii Oud. has been reported to be a potential antagonist of *Ustilago segetum* (Pers.) Roussel *tritici* Jensen, the causal organism of loose smut of wheat[1]. Earlier, Aggarwal *et al.*[2] reported that *T. viride*, *Bacillus subtilis* and *Gliocladium deliquescens* suppressed the expression of loose smut infection in the field. One of the prerequisites for the rational utilization of the biological properties of a potential antagonist is an understanding of the mechanisms underlying the mycoparasitic process. We report here the mechanism of interaction between *T. koningii* and *U. segetum tritici* through scanning electron microscopy (SEM).

One ml chlamydospore suspension of *U. segetum tritici* prepared in sterilized water having $15 \times 10^5 - 25 \times 10^5$ spores/ml was poured into Petri plates containing 2% plain agar. The inoculated plates were incubated at 20 ± 1°C for 2 days. The germinated chlamydospores were marked and transferred to potato dextrose agar (PDA) slants and maintained at 20 ± 1°C. *T. koningii* was isolated from wheat rhizospheric soil by dilution plate method and maintained in PDA slants.

Hyphal interaction between *U. segetum tritici* and *T. koningii* was studied by dual culture test. An agar disc containing mycelium of the pathogen was inoculated in one corner of PDA-poured Petri plate and incubated for 3 weeks at 20 ± 1°C. The agar disc containing actively growing *T. koningii* culture was inoculated on the other side of plate and incubated at 20 ± 1°C for 3–4 days. Mycelial samples from the interaction region were cut by a cork borer and processed for scanning electron microscopy.

Mycelial samples from the interaction zone were vapour fixed with 2% (w/v) osmium tetraoxide in distilled water for 20 h at room temperature, dried in a desiccator over $CaCl_2$ for 24 h and

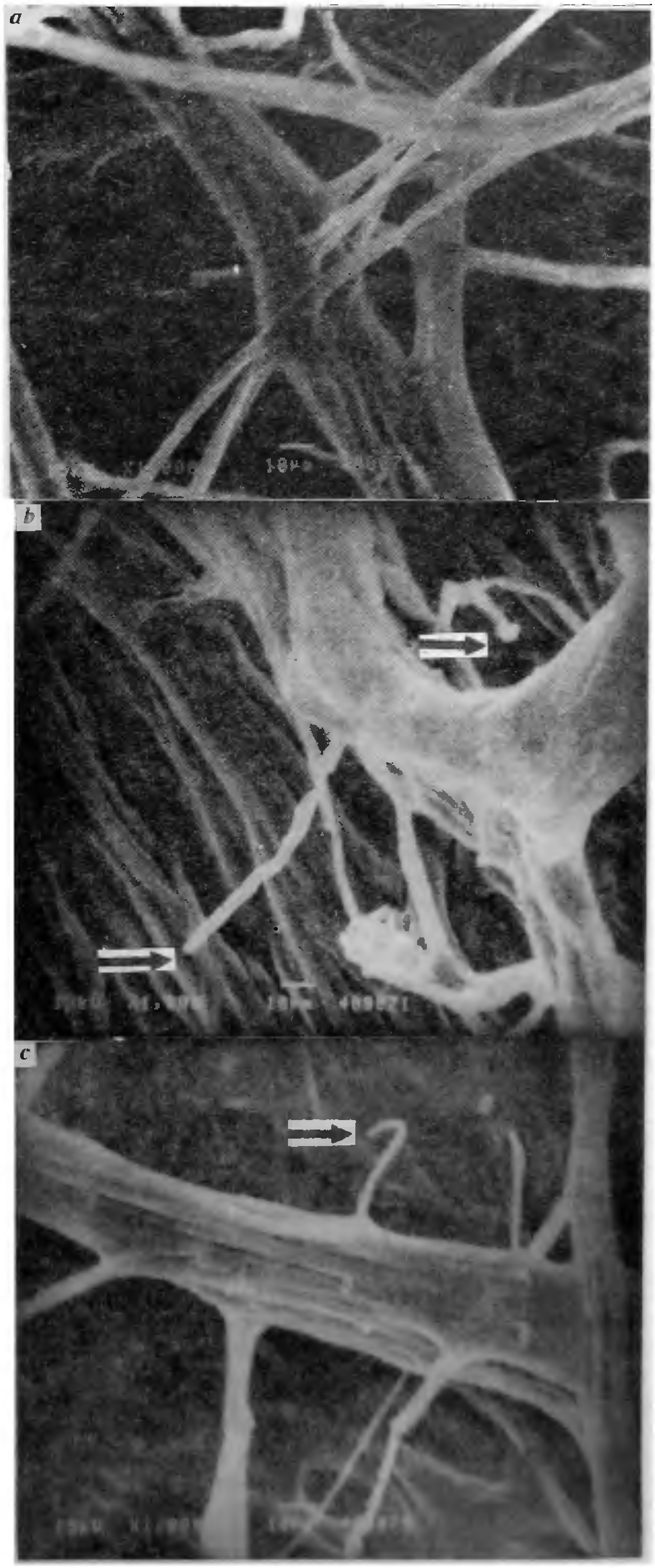

Figure 1*a*–*c*. Hyphal interaction between *Trichoderma koningii* and *Ustilago segetum tritici* as seen through SEM. ***a***, Showing overgrowth; ***b***, Showing penetration pegs; ***c***, Showing hook-like penetration pegs.

sputter-coated with gold in a sputter coater (JE 1100). Samples were kept in the desiccator until examination by SEM (JEOL, JSM 5200) at 20 kV and micrographs were taken.

Mycelium of *U. segetum tritici* was totally overgrown by *T. koningii*, which sporulated abundantly after 4 days. SEM observations also showed overgrowth of *T. koningii* on the hyphae of *U. segetum tritici* (Figure 1 *a*). The antagonist formed penetration pegs with slightly enlarged tips at a few places (Figure 1 *b*). Sometimes hook-like projections were also seen penetrating the host mycelium (Figure 1 *c*). The mycelium of *U. segetum tritici* was suppressed and lysed. The collapse and lysis of host pathogen may be attributed to the production of antifungal substances. The disorganization of the host cells and concomitant changes in the cells of the parasite have been established for *Trichoderma* spp. and *Gliocladium* spp[1,3]. Benhamou and Chet[4] demonstrated through SEM that coiling of the antagonist (*T. harzianum*) around its host (*R. solani*) was an early event preceding hyphal damage.

The present study revealed that the physical contact between the two fungi followed by lysis of the host cells seems to be the mechanism of biological control of *Ustilago segetum tritici*.

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Ecology of forest fires in chir pine (*Pinus roxburghii* Sarg.) forests of Garhwal Himalaya

Research over the last five decades has proved fire as a part of normal 'climate' in most of the terrestrial ecosystems of the world. Therefore, biotic communities adapt to and compensate for this integral factor, just as they do for other ecological factors[1], e.g. temperature, rainfall, humidity, etc. It is believed that if properly used fires can be a management tool of great value. The effects of fire on vegetation depend on a number of factors, e.g. topography of the landscape, the structure of vegetation, fire intensity and its mode of spread, types of fuel and fuel loading, season of burning, post fire precipitation etc.

In any terrestrial ecosystem fire can be of natural origin (lightning, volcanic eruption, spontaneous combustion and spark produced by rock slides) or man-caused[2,3]. Of the total incidents of forest fire in this Garhwal Himalayan region, 63% were man-caused intentional and remaining 37% were accidental[4–6]. Intentional fire is started traditionally by the natives in the pine forests and associated grazinglands mostly during summer season to enhance forage. In addition to this, graziers, school boys, forest contractors and forest personnel for various reasons cause intentional forest fire. Among the important causes of accidental forest fire are fire escaped during burning of crop remains from the agricultural fields, flames escaped from fire lines during controlled burning by forest department, a live cigarette or bidi butt thrown by a careless passerby, motor road repairs, cooking and campfire activities, respectively, of pilgrims and tourists etc[4–6].

Among the two types of forest fire viz., surface fire and crown fire or wild fire[1], most of the pine and other forests experience surface fire in the Garhwal Himalaya. The total burnt area under an event of forest fire seldom exceeds 4–5 km^2 in any pine forest stand of this region. Usually the frequency of fire in the forests has been observed to be 2–5 years while 11% pine forests of the region experience fire annually[5,6]. Surface fire follows two main mode of spreading. It may travel down from hill top to hill base, generally at a slow pace, or it may ascend up from hill base to top. The ascending blaze spreads at high speed (difficult to control) but the effects on vegetation generally remain lower. However, sometimes ascending flames culminate in severe crown fire.

In Garhwal Himalaya forests situated between 300 m and 2000 m above m.s.l. can be considered as fire prone. The region between 1000 and 1800 m above m.s.l. is mainly dominated by chir pine (*Pinus roxburghii*), forests representing densely populated zone of the region. People derive fodder and other forest produce for their subsistence living from the surrounding chir pine forests. The pine forests having relatively thin canopy provide congenial environment for the growth of herbaceous vegetation comprising grasses, sedges, legumes and other non leguminous species. The herbaceous vegetation shows maximum diversity under pine forests than any other forests of the region[7]. The average peak herbaceous biomass production (used as fodder) from the chir pine forests of this region has been estimated as much as 3.31 t per ha[7]. The pine needles are collected for animal bedding and are used as manure after decomposition. The average litterfall from the chir pine forests has been estimated as 8.5 t per ha per year[7]. Besides, timber, tylosed wood, tree bark, fuel wood, etc. are also collected from the pine forests.

Pine forests of the world, African savannas and Mediterranean shrublands are considered as fire adapted/dependent ecosystems because their continued existence depends on the periodic occurrence of fire[2]. In this re-