2. REVIEW OF LITERATURE

The information on various aspects of *Trichoderma* in view of growth and other activities are reviewed as following:

1. Distribution

Soderstrom (1975) reported vertical distribution of micro fungi in a spruce soil in south of Sweden, soil-washing technique was used for the isolation of micro fungi from soil of a planted Norway spruce forest in the south of Sweden. About ninety different fungal species were identified. The most frequently isolated genera were *Mortierella* (represented by 16 species), *Penicillium* (13 species), and *Trichoderma* (4 species), which together constituted 71% of the total number of isolates (*Mortierella* 41%, *Penicillium* 19%, *Trichoderma* 11%). A significant (*P < 0.01*) difference in abundance between the different soil horizons was found for twenty-three species. Out of these surface litter layer and decaying horizon layers *Mortierella ramanniana* and *Trichoderma polysporum* were very abundant and in decaying horizon. *T. viride* and *Aeremonium griseoviride* reached high frequencies.

2. Environmental conditions

Hannusch and Boland (1996), worked on interactions of air temperature, relative humidity and biological control agents on gray mold of bean. The interactions of *Botrytis cinerea* and seven biological control agents (BCAs) were examined in controlled environments to determine the influence of selected relative humidity (RH 90, 95 and 100%) and air temperatures (20, 24 and 28 °C) on gray mold of bean. All main effects and interactions were significant (*P < 0.05*) among the 72 treatments. In the control, lesions of gray mold developed under different environmental conditions but were largest at 24 °C x 95 and 100% RH, and 28 °C x 95% RH. Interactions of environment, BCAs and gray mold were complex.
*Alternaria alternata*, *Drechslera* sp., *Myrothecium verrucaria*, *Trichoderma viride*, *Gliocladium roseum* and an unidentified pink yeast were all highly dependent on environment for biological control efficacy, and changes of 4°C or 5% RH were associated with variability in disease and suppression ranged from ≤ 15 to 100%. Efficacy of *Epicoccum purpurascens* appeared independent of environment and biological control agent suppressed disease by 100% in all environmental treatment. Suppression of Gray mold by many BCAs was most effective under environmental conditions least conducive to disease. Therefore, evaluation of potential BCAs in environmental condition that are marginal for disease can over estimate their efficacy in field environmental. Assessment of biological control efficacy in various environment can be used to more accurately assess the potential of biological control agents.

### 3. Seasonality

Widden and Abitol (1980), conducted experiment to investigate the effects of seasonal change on *Trichoderma* populations in a spruce forest soil. Nine species of *Trichoderma* were isolated, of which two are undescribed species. Out of the species, which occurred at relatively high frequencies, *T. polysporum* was most abundant in winter, *T. viride* in the spring and *T. koningii* were most abundant in the summer month. All species showed a high level of spatial variation at all times of the year. A multiple-regression analysis, relating the occurrence of the *Trichoderma* species to measured environmental parameters indicated that, for *T. viride*, there had a significant relationship between its occurrence, and soil moisture content and temperature. For the other three common species, the best predictive equations incorporated biotic variables, mainly the occurrence of other *Trichoderma* species. It is, therefore, suggested that the seasonal variation in the occurrence of *Trichoderma* is mediated to a large extent by competition with other species rather than by the direct effect of a biotic factors.

Widden and Hsu (1987), evaluated five species of *Trichoderma* which colonize maple and pine litter under a range of temperature conditions.
Widden and Abitol (1980) studied the seasonal effect on the distribution of *Trichoderma*. Temperature, soil, moisture and competition from different *Trichoderma* play a significant role in the distribution of *Trichoderma*. *T. polysporum* was most abundant in the fall and winter, *T. viride* in the spring and *T. koningii* was most abundant in the summer month. *In vitro* effect of temperature, pH and water potential for *G. virids* and three isolates of *Trichoderma*, needed to evaluated in the field where wide varieties of those factors exist (Jackson *et al.*, 1991; Knudsen and Bin, 1990, Summerell and Burgess, 1989). For quantitative description of growth or activity development of models are essential. Ratkowsky *et al.* (1983) developed a model to describe the growth of bacteria for different temperatures. The non-linear model uses four parameters in which two parameters represent minimum and maximum temperature respectively. Cuppers *et al.* (1997) modeled growth of *T. harzianum* and other spoilage causing organisms at various temperature and salt concentrations. Rosso *et al.* (1993) proposed model of temperature dependence that uses three cardinal temperatures (*Tmin*, *Tmax*, and *Topt*) and specific growth rate at the optimum temperature (*Topt*). Effect of salt concentration on fungal growth may be linked to soil metric potential of the soil where activity of the fungus is under consideration.

4. Survival

Summerell and Burgess (1989), studied the factors influencing survival of *Pyrenophora tritici-repentis*. They found that water potential and cool dry conditions favored the survival of *Pyrenophora tritici-repentis* in artificially colonized wheat chaff stored with and without soil contact. *P. tritici-repentis* was recovered after 104 wk from wheat chaff stored at approx. -150 MPa at 10°, 20° and 30 °C, but the fungus was not recovered after 26 wk at -19.5, -2.8 and 0 MPa at 30°C. Recovery of the fungus from chaff in contact with soil was less than that from chaff with no soil contact. At water potentials higher than -39 MPa, *P. tritici-repentis* was initially displaced by *Pythium oligandrum* at 10°, and *Aspergillus terreus* and *Chaeomium globosum* at 20° C and 30° C, which in turn were succeeded
conditions with cellobiose carboxymethyl cellulose as sole source of carbon instead of using them as wild collection (directly from soil) and can be suitable explored for biocontrol.

5.1. Techniques

Soderstrom (1975), studied vertical distribution of micro fungi in a spruce soil in south of Sweden. He found soil-washing technique was best used for the isolation of micro fungi from soil of a planted Norway spruce forest in the south of Sweden. About ninety different fungal species were identified. The most frequently isolated genera were Mortierella, Penicillium, and Trichoderma, which together constituted 71% of the total number of isolates. A significant ($P < 0.01$) difference in abundance between the different soil horizons was found for twenty-three species.

6. Medium

Elad, Chet and Henis (1981), working on selective medium for improving quantitative isolation of Trichoderma species from soil, developed a Trichoderma-selective agar medium (TSM). It was developed for quantitative isolation of Trichoderma spp. from soil. Selectivity was obtained by using chloramphenicol as a bacterial inhibitor, and p-dimethyl aminobenzene, diasodium sulfonate and rose-bengal as selective fungal inhibitors. The TSM also contains a low concentration of glucose, which allows relatively rapid growth, and sporulation of Trichoderma which enables convenient and rapid identification of the colonies. All the 15 Trichoderma isolates tested formed colonies and grew well on this medium. Recovery of Trichoderma from artificially inoculated soils was high and was not affected by soil type or by other microorganisms. A positive correlation was observed between Trichoderma added to soil and counts of Trichoderma colonies on TSM plates. When combined with a soil pellet sampler, the selective medium was also used successfully for recovery of the indigenous Trichoderma population of natural soils.
Papavizas and Lumsden (1982) adopted *Trichoderma* medium (TME) for selective isolation from soil. *Trichoderma* selective media (TSM) for quantitative isolation from soil (Elad and Chet, 1983; Elad et al., 1981), gave superior results than TME (Saha et al., 1997). TSM is further improved by adding Captan 50% 10 mg/L (TSMC) to avoid some common contaminants like *Mucor, Rhizopus, Penicillium* especially *Fusarium* species (Askew and Lang, 1993). Kuling et al. (2000) isolated *Trichoderma* spp. from Himalayan soils using PDA and cellulose agar.

Nelson, and Hoitink (1983) while studying the role of microorganisms in the suppression of *Rhizoctonia solani* in container media amended with mature (>11-wk-old) composted hardwood bark (CHB) was eliminated by heat (60 C) and gamma radiation (275 krad). Media amended with green (<11-wk-old) CHB were only slightly suppressive and heating had no effect on the resulting incidence of damping-off. Suppression could be reestablished in heated mature CHB media by adding 10% (v/v) unheated mature CHB or $10^7$ colony-forming units (CFU) of *Trichoderma harzianum* per gram of container medium. Propagule of *Rhizoctonia solani* were killed rapidly in unheated media containing mature CHB and in heated mature CHB media amended with *T. harzianum* but a high percentage of propagule remained viable in media amended with either green or heat-treated mature CHB. Results suggest that suppression of *Rhizoctonia* (damping-off of radish) in container media amended with mature CHB is induced by microbial activity. Whereas the low level of suppression observed in green CHB amended media may involve chemical inhibitors.

7. Temperature vs. Biocontrol

Transmo and Dennis (1978), studied the effect of temperature on antagonistic properties of *Trichoderma* species. Isolates from different species-groups were tested for growth, production of non-volatile inhibitors, volatile inhibitors and hyphal interaction against *Botrytis cinerea* Pers. ex Fr. and *Mucor mucedo* Fr. at 5, 10 and 20 °C. Most isolates tested were antagonistic against the test fungi. Inhibition of growth by non-volatile
metabolites from *Trichoderma* species was most severe at the lowest temperature, whereas more isolates produced volatile inhibitors at higher temperatures. No effect of temperature was observed on the hyphal interaction. Contrary to a previous report it was shown that carbon dioxide did not account for the inhibition by the volatile component. Mahrer (1979), developed a model for prediction of soil temperatures of a soil mulched with transparent polyethylene. The model takes into consideration environmental condition as well as physical characteristics of both the mulch material and the soil. The ability of the model to predict the temperature of the mulched and unmulched soil is tested. It is shown that soil temperatures of a wet mulched soil are significantly increased, primarily due to the elimination of evaporation and partly due to the greenhouse effect of the polyethylene film. In the case of a dry mulched soil the greenhouse effect is dominant. Thus a smaller temperature increase is obtained.

7.1. Temperature vs. cellulytic activity

Jotul and Singh (1985), worked on temperature relation and cellulytic activity of weed fungi occurring in the beds of *Pleurotus sajo-o- caju* (Fr.) Singh and Sandhu (1985) found that nine species of fungi were isolated from beds of *Pleurotus sajo-o- caju*. Among these five species namely *Curvularia lunata, Fusarium graminearum, Gliocladium virens, Rhizopus arhizus, Trichoderma viride* were mesophilic; *Aspergillus fumigatus* was thermo tolerant and *Malbranchea pulchella* var. *sulphurea, Myriococcum albomyces, Thermomyces lanuginosus (Humicola lanuginosus)* were thermophilic. All these species when tested showed cellulytic activity except for *Malbranchea pulchella* var. *sulphurea* and *Thermomyces lanuginosus* both of which happened to be thermophilic. The Cellulolytic species were able to degrade filter paper as well as to hydrolyze the carboxyl-methyl-cellulose (CMC) solution. *Aspergillus fumigatus* degraded the filter paper in highest degree while the activity of enzyme (represented by loss of viscosity of CMC solution) was highest in cell free culture filtrate of *Trichoderma viride*. 
Singh and Sandhu (1985) worked on thermophilous fungi in Port Blair soils. They collected soils from eight different sites of saline marshy soils of Port Blair situated in the Andaman, and Nicobar islands, India. A total of 93 333 colony-forming units representing 46 species of thermophilous fungi were isolated by the soil dilution method. The fungi were tabulated in order of ecological importance based on their frequency, relative density, and their presence during the selected sites of this study. This is apparently the first study of these fungi from Port Blair soils. Out of these 46 species, *Mucor hiemalis*, *Rhizopus oligosporus*, *Thermoascus crustaceous*, *T. thermophilous*, and *Trichoderma pseudokoningii* are new records for India

Duck, Jeffrey and Montenecourt (1986), studied temperature sensitive mutant of *Trichoderma reesei* in secretion of cellulose derived from hyper secretary strain RL-P37 were isolated and characterized. Compared to the parent strain, one mutant (LU-ts 1) grew well in the mycelia phase at both permissive (25°C) and nonpermissive (37°C) temperatures. However, the secretion of overall protein and active cellulases was significantly reduced in the mutant at the higher temperature. No accumulation of active cellulases or intracellular proteins was observed in the mycelia of LU-ts 1 at 37°C. The inhibitory effects of temperature on cellulose secretion in LU-ts 1 were reversible. Isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis analyses confirmed that the secretion of the major cellulases was greatly reduced in LU-ts 1 at 37°C. Molecular characterization of the various temperatures sensitive secreting mutants of *T. reesei* should help elucidate the crucial aspects of the secretary pathway of this cellulolytic fungus.

7.2. Temperature, distribution

Widden and Hsu (1987) evaluated five species of *Trichoderma* to colonize maple and pine litter under a range of temperature conditions. Widden and Hsu, (1987) working on competition between *Trichoderma* species for effect of temperature and litter type observed the ability of five
species of Trichoderma to competitively colonize maple and pine litter under a range of temperature conditions. It was evaluated and compared with published data on competition for spruce litter. It was shown that generally T. polysporum and T. viride competed most effectively under low temperature conditions (5-15°C), whereas T. hamatum, T. koningii, and an undescribed species, LP58, competed best at higher temperatures (20-25°C). Of the five species tested, T. koningii was an effective competitor over a wide range of temperatures, whereas LP 58 was not, very effective under any of the experimental conditions. Litter type did not have much effect on the competitive ability of T. koningii or T. polysporum, T. hamatum tended to compete effectively over a wide range of temperatures for maple litter, and was a poorer competitor for pine litter than for maple litter at 25°C. Trichoderma sp. LP58 was a better competitor for maple litter than for pine litter. T. viride was shown to be a very poor competitor for maple litter, even at low temperatures. The competitive abilities of T. hamatum, T. koningii; T. polysporum and T. viride, under the experimental conditions, appear to reflect the trends that emerge from field observations, whereas the data for LP58 conflict with field observations. These observations would suggest that the niche factors influencing the distribution of LP58 are somewhat different from those influencing the other species investigated.

Widden, Cunningham, and Brenda, (1989) made studies on decomposition of cotton by Trichoderma species: influence of temperature, soil type and nitrogen levels and found the effect of temperature on the ability of five species of Trichoderma to decompose cellulose which was evaluated in three types of sterilized soils. The loss in tensile strength of cotton strips was used as an index of cellulose decomposition. The influence of levels of nitrogen on cotton degradation was also evaluated using silica sand as a substitute for soil. A greater loss in cotton tensile strength was obtained with T. virens and T. viride than with T. hamatum, T. polysporum, or T. koningii. All species responded similarly to nitrogen levels and temperature, with decreased activity at low nitrogen and low temperature. The soil also had a large influence on the rate of decomposition of the
strips. The soil from a maple forest gave rise to greater losses in tensile strength than did soil from a white pine or Norway spruce plantation. These results emphasize the importance of the soil Chemistry in determining fungal activities in the field, and the difficulties of extrapolating results of cellulose utilization studies in the laboratory to the field.

Knudsen and Bin, Li (1990), working on the effects of temperature, soil moisture, and wheat bran on growth of *Trichoderma harzianum* from alginate pellets recorded radial growth rates and hyphal densities were quantified for hyphae originating from alginate pellets containing hyphae of the biocontrol fungus *Trichoderma harzianum*. Pellets containing 1.8 mg of hyphal biomass (mean dry weight) and either 0 or 2.5 mg of wheat bran were buried beneath sheets of nylon mesh in a steamed silt loam soil, then incubated at 15,20, or 25 C and -0.03, -0.1, or -0.5 MPa soil metric potential. Extent of hyphal growth was visually mapped after 0, 1, 2, 3, 5, 7, and 14 days, and mean radii were calculated from digitized maps. A visual assessment key for hyphal density was developed with a computer graphics program. With the key, hyphal density at 5, 10, 20, 30, and 40 mm from pellets was quantified after 7 and 14 days. For radial extension of hyphae, a logistic growth model was fit, with the assumption of a 24-hr lag (germination) period (mean R2 for all treatments). Multiple regressions of environmental variables against the slope of each growth curve showed that temperature had a significant positive effect on radial growth rate but that metric potential and bran effects were not significant. Hyphal density declined exponentially with distance from pellets. Hyphal density was significantly higher with bran, in drier soil and after 14 versus 7 days. Temperature did not significantly affect hyphal density at 7 or 14 days.

Methods and results presented may be useful for comparison of nutritional additives or other formulations for palletized biocontrol fungi or as a basis to develop predictive models for performance of palletized biocontrol fungi at different application rates and under varying environmental conditions.

Hannusch and Boland, (1996), worked on interactions of air, temperature, relative humidity and biological control agents on gray mold
of bean. They examined the interactions of *Botrytis cinerea* and seven biological control agents (BCAs) in controlled environments to determine the influence of selected relative humidity's (RH) (90, 95, and 100%) and air temperatures (20, 24 and 28 °C) on gray mold of bean. All main effects and interactions were significant (P<-.05) among the 72 treatments. In the control, lesions of gray mold developed under all environmental conditions but were largest at 24 °C x 95 and 100% RH, and 28 °C x 95% RH. Interactions of environment, BCAs and gray mold were complex. *Alternaria alternata*, *Drechslera* sp., *Myrothecium verrucaria*, *Trichoderma viride*, *Gliocladium roseum* and an unidentified pink yeast were all highly dependent on environment for biological control efficacy, and changes of 4 °C or 5% RH were associated with variability in disease; suppression that ranged from 15 to 100%. Efficacy of *Epicoccum purpurascens* appeared independent of environment and this BCA suppressed disease by 100% in all the environmental treatments. Suppression of gray mold by many of the BCAs was most effective under environmental conditions least conducive to disease. Therefore, evaluations of potential BCAs in environmental conditions that are marginal for disease can overestimate their efficacy in field environments. Assessments of biological control efficacy in various environments can be used to more accurately assess the potential of BCAs.

7.3. Temperature and Salt

Cuppers, Oomes, and Brul, (1997) developed a model for the combined effects of temperature and salt concentration on growth rate of food spoilage molds on a solid culture medium at various temperatures and NaCl concentrations by using five common food spoilage molds (*Penicillium roquefortii*, *Trichoderma harzianum*, *Paecilomyces variotii*, *Aspergillus niger*, and *Emircella nidulans*). For the description of the growth rate (expressed as the increase in colony diameter per unit of time) as a function of temperature and NaCl concentration, a six-parameter model has been developed. The model combines either the Russo-type or the Ratkowsky-type temperature dependence with the NaCl concentration dependence.
derived from the relationship between the growth rate and their water activity, as proposed by Gibson and coworkers (1994). The model will be of use to food microbiologists whose aim is to predict the likelihood of fungal spoilage.

8. Model

Mahrer (1979), developed prediction of soil temperatures of a soil mulched with transparent polyethylene. The model takes into consideration environmental condition as well as physical characteristics of both the mulch material and the soil. The ability of the model to predict the temperature of the mulched and unmulched soil is tested. It is shown that soil temperatures of a wet mulched soil are significantly increased, primarily due to the elimination of evaporation and partly due to the greenhouse effect of the polyethylene film. In the case of a dry mulched soil the greenhouse effect is dominant. Thus a smaller temperature increase is obtained. For quantitative description of growth or activity development of models are essential.

Ratkowsky et al (1983) developed a model to describe the growth of bacteria for different temperatures. The non-linear model uses four parameters in which two parameters represent minimum and maximum temperature bound respectively.

Cuppers et al. (1997) modeled growth of *T. harzianum* and other spoilage causing organisms at various temperature and salt concentrations.

Rosso et al. (1993) proposed model of temperature dependence that uses three cardinal temperatures ($T_{\text{min}}$, $T_{\text{max}}$ and optimal growth temperature $T_{\text{opt}}$) and specific growth rate at the optimum temperature ($r_{\text{opt}}$). Effect of salt concentration on fungal growth may be linked to soil metric potential of the soil where activity of the fungus is under consideration.

Ratkowsky et al. (1983) while working on model for Bacterial culture growth rate throughout the entire biokinetic temperature range found that the square-root relationship for modeling the growth rate of bacteria below the optimum growth temperature was extended to cover the full biokinetic
temperature range. Two of the four parameters of this new nonlinear regression model represent minimum and maximum temperature bounds, respectively, for the predicted growth of the culture. The new model is easy to fit and has other desirable statistical properties. For example, the least-squares estimators of the parameters of the model were almost unbiased and normally distributed.

Russo et al. (1995) working on convenient model to describe the combined effects of temperature and pH on microbial growth recorded maximum microbial specific growth rate was described as a function of pH and temperature is presented. The seen parameters of this model are the three cardinal pH parameters (the pH below which no growth occurs, the pH above which no growth occurs, and the pH at which the u_max is optimal), the three cardinal temperature parameters and the specific growth rate at the optimum temperature and optimum pH. The model is a combination of the cardinal temperature model with inflection and the cardinal pH model (CPM). The CPM was compared with the models of Wijtzes et al. and Zwietering et al. by using previously published data set. The models were compared on the basis of the usual criteria (simplicity, biological significance and minimum number of parameters, applicability, quality of fit, minimum structural correlations, and ease of initial parameter estimation), and our results justified the choice of the CPM. The combined model was constructed by using the hypothesis that the temperature and pH effects on the maximum are independent. An analysis of this new model with an Escherichia coli showed that there was a good correspondence between observed and calculated values.

Cuppers et al. (1997) showed the model for the combined effects of temperature and salt concentration on growth rate of food spoilage molds. The model was developed on a solid culture medium at various temperatures and NaCl concentrations by using five common food spoilage molds (Penicillium roquefortii, Trichoderma harzianum, Paecilomyces variotii, Aspergillus niger, and Emerecella nidulans). For the description of the growth rate (expressed as the increase in colony diameter per unit of time) as a
function of temperature and NaCl concentration, a six-parameter model has been developed. The model combines either the Rosso-type or the Ratkowsky-type temperature dependence with the NaCl concentration dependence derived from the relationship between the growth rate and their water activity, as proposed by Gibson et al. (1987).

9. Spatial arrangements

Campbell and Noe (1985) described that collection of organisms has spatial arrangements which may help in the elucidation of the organism’s ecology. Spatial pattern of any organism provides an opportunity to characterize its attributes from a series of static samples over the time. The characterization of a spatial pattern thus provides a tool for the development of plausible biological and environmental hypotheses to account for the association among species and the behavior of specific organisms.

10. Composting

Verma et al. (1999) described that three levels of cage system poultry waste (0, 10 and 20% of the substrate) with and without the compost activator, Trichoderma harzianum were used for composting. The temperature was higher at periphery than that at the center of all the heaps up to 3 weeks of heaping, irrespective of treatments. The temperature difference was as high as 10-15°C in heaps prepared from soybean trash and 5-10°C in heaps prepared from rice straw. Within 48 hr of heaping maximum temperature of 52.5 and 61°C was recorded in rice straw + soybean trash and soybean trash heaps respectively. After 3 weeks, the temperature at periphery declined sharply and stabilized and remained almost equal to the temperature at the center of the heaps up to 8 weeks. With time, shrinkage along the periphery was found more in rice straw heaps forming a hump structure. Activator did not cause shrinkage significantly. Nutritionally, soybean trash yielded the best quality compost. The increased level of poultry waste increased the nutrients status marginally. Soybean trash offered better support for Azotobacter than rice.
straw. Lower C: N ratio in compost from soybean trash attributed to higher population of *Azotobacter*.

11. Decomposition

Widden, Cunningham, and Brenda, (1989) made studies on decomposition of cotton by *Trichoderma* species and found that the effect of temperature on the ability of five species of *Trichoderma* to decompose cellulose was best in three types of sterilized soils. A greater loss in cotton tensile strength was obtained with *T. viride* and *T. virens* than with *T. hamatum*, *T. polysporum*, or *T. koningii*. The soil also had a large influence on the rate of decomposition of the strips.

12. Cellulose

Henderson, McDonald and Anderson (1982) worked on the effect of a cellulose preparation derived from *Trichoderma viride* on the chemical changes during the ensilage of grass, lucerne and clover. They found that ryegrass, lucerne and clover treated with formic acid [4.5 liter/ton of crop] + *Trichoderma* cellulose preparation (commercial) [4 g/Kg] at 0, 15, 35 and 50°C resulted in more hydrolysis in grass that in legume silage. These experiments were conducted in test tube with finely chopped silage minced together. The result showed that the greatest losses of cellulose occurred at 15 and 35°C on minced silage.

13. Osmotic coefficient

Lang (1967), while working on osmotic coefficient and water potential of sodium chloride solutions from 0 to 40°C, calculated values of the water potentials of aqueous sodium chloride at concentrations of 0.05 to 2 molar, and temperatures from 0 to 40°C. Experiments necessary to determine these water potentials were done long ago in the study of electrolyte solutions. Thus, except at 25°C or for limited intervals of concentration of water potentials via the osmotic coefficients were not readily obtainable. Such values are required when calibrating psychomotor used for *in situ*
measurement of water potentials in plants and soil. For these measurements of water potential the temperature is not constant, so calibrations must be made at various temperatures. The water potential considered here is the difference between the partial free energy of water in the system, that of a pool of water at atmospheric pressure at the same temperature and elevation as the water in the system. The water potential is related to the relative vapor pressure of the water in the system.

14. Osmotic coefficient and Water potential

Michel and Kaufman (1973) found that osmotic potential of aqueous solutions of polyethylene glycol 6000 (PEG-6000) was curvilinear related to concentration. At given concentrations, increased linearly with temperature. The effects of concentration and temperature on PEG-6000 solutions differ from those for most salts and sugars and apparently are related to structural changes in the PEG polymer. Observation with thermocouple psychrometer are more negative than those with a vapor pressure osmometer, with the psychrometer probably giving the more correctly for the bulk solutions. Viscometer and gravimetric analysis are convenient methods by which the concentrations of PEG-6000 solutions can be measured.

15. Plant Growth

Windham, Elad and Baker (1986). While studying a mechanism for increased plant growth induced by Trichoderma spp confirmed that, enhanced plant growth results from amendments of soil with Trichoderma harzianum and T. koningii. It was investigated to determine if increased growth could be attributed to a direct effect of these Trichoderma spp. on the plant or a secondary effect due to control of minor plant pathogens. Addition of Trichoderma spp. to autoclaved soil increased rate of emergence of tomato and tobacco seedlings over that of the controls. Eight weeks after planting, root and shoot dry weights of tomato and tobacco were increased 213-275 and 259-318% respectively, over the controls. When population densities of soil micro flora (other than Trichoderma spp.) were determined,
no quantitative and qualitative differences were observed between soils infested with *Trichoderma* spp. and the controls. When soil fertility was increased, the level of increased tomato growth induced by *Trichoderma* spp. was enhanced. Radish plants grown with *T. harzianum* were larger than radish plants grown under similar conditions without the agent. The rate of seed germination was increased compared with controls where *Trichoderma* spp. was not present when seeds were separated from *Trichoderma* spp. by a cellophane membrane. It can be concluded that these *Trichoderma* spp. produce a growth-regulating factor that increases the rate of seed germination and dry weight of shoots and stems.

16. Shelf life

Nakkeeran, Sankar, and Jeyarajan (1997) standardized the storage conditions to increase the shelf life of *Trichoderma*. The formulations were studied. Vermiculite bran acid fermentor biomass (VBA-FB) of *T. viride* recorded the highest mean population in milky white bag (205 x 10^6 cf/g). The population of antagonist was in its exponential phase (309 x 10^6 cf/g) till 30 days after storage. Irrespective of the bag color, the steady increase in the population level was seen after 30 days of storage. Studies on storage temperatures revealed that 20-30°C was optimum to store VBA-FB formulation in milky white bags. At this range of temperature, even after 75 days, the product contained 206-271 x 10^6 cf/g. In talc based formulation there was a slow decline in the population of *Trichoderma* due to storage. The mean population of 230-242 x 16 cf/g was observed in talc-based formulation at 20-30°C after 75 days of storage. This indicates that the product could be safely stored as talc based formulations in milky-white bags at room temperature (20-30°C) for up to 75 days.

17. Biocontrol

Elad, Chet, Katan (1980) found an isolate of *Trichoderma harzianum* capable of lysing mycelia of *Sclerotium rolfsii* and *Rhizoctonia solani* from a soil naturally infested with those pathogens. In culture, *T. harzianum* grew better than *S. rolfsii* and invaded its mycelium under growth conditions
adverse to the pathogen; e.g. high penta- chloro- nitrobenzene concentrations, high pH levels, or low temperatures. Under greenhouse conditions, incorporation of the wheat-bran inoculums preparation of *T. harzianum* in pathogen-infested soil significantly reduced bean diseases caused by *S. rofssii*, *R. solani*, or both, but its biocontrol capacity was inversely correlated with temperature. The wheat bran preparation of *T. harzianum* increased growth of bean plants in a non-infested soil and it controlled *S. rofssii* more efficiently than a conidial suspension of the same antagonist. An uninoculated wheat bran preparation increased disease incidence. In naturally infested soils, wheat bran preparations of *T. harzianum* inoculums significantly decreased diseases caused by *S. rofssii* or *R. solani* in three field experiments with beans, cotton, or tomatoes, and they significantly increased the yield of beans.

Mechanisms for biocontrol by *Trichoderma* are antibiotics, lyses, competition and mycoparasites (Cook and Baker, 1983. Papavizas and Lumsden. 1980). Because of the strong cellulolytic activity exhibited by many *Trichoderma* species, it is natural to assume that celluloses may play an important role in their ecology. Different species have shown to have distinct environmental preferences (Danielson and Dave 1973a &b; Widden 1986a. 1986b; Widden, 1979) and to differ in their relative competitive abilities depending on environmental temperatures (Widden 1984) and on the nature of the substrate (Widden and Hsu 1987). One potential mechanism which determine the absence of competition under differing environmental conditions may be differential influence and/or vegetation history of the soil on the ability of different species to break down cellulose.

*Widden et al (1989) showed cellulose activity in a similar fashion to nitrogen and temperatures. Activity decreased with at low nitrogen and low temperature. Nelson and Hoitink (1983) while studying the role of
undescribed species, LP58, competed best at higher temperatures (20-25°C). Of the five species tested, T. koningii was an effective competitor over a wide range of temperatures, whereas LP 58 was not, very effective under any of the experimental conditions. Litter type did not have much effect on the competitive ability of T. koningii or T. polysporum, T. hamatum tended to compete effectively over a wide range of temperatures for maple litter, and was a poorer competitor for pine litter than for maple litter at 25°C. Trichoderma sp. LP58 was a better competitor for maple litter than for pine litter. T. viride was shown to be a very poor competitor for maple litter, even at low temperatures. The competitive abilities of T. hamatum, T. koningii; T. polysporum and T. viride, under the experimental conditions, appear to reflect the trends that emerge from field observations, whereas the data for LP58 conflict with field observations. These observations would suggest that the niche factors influencing the distribution of LP58.

O’Neill et al. (1996) worked on biological control of Botrytis cinerea on tomato stem wounds with Trichoderma harzianum and found the effectiveness of Trichoderma harzianum in suppression of tomato stem rot caused by Botrytis cinerea. It was examined on tomato stem pieces and on whole plants. Ten days after simultaneous inoculation with B. cinerea and T. harzianum, the incidence of infected stem pieces was reduced by 62-84%, the severity of infection by 68-71% and the intensity of sporulation by 87%. The biocontrol agent did not reduce the rate of rotting once infection was established. However, sporulation by B. cinerea was specifically reduced on these rotting stem pieces. Temperature had a greater effect than vapor pressure deficit (VPD) on the efficacy of biocontrol. Suppression of B. cinerea incidence by T. harzianum on stem pieces was significant at 10°C and higher temperatures up to 26 °C. Control of infection was significantly lower at VPD of 3 kPa (60% reduction), than at VPD < 1.06 kPa (90-100% control). Reductions in the severity of stem rotting and the sporulation intensity of gray mold were generally not affected by VPD in the range 0.59-1.06 kPa. Survival of T. harzianum on stems was affected by
both temperature and VPD and was greatest at 10 °C at a low VPD and 26 °C at a high VPD.

Todkar et al. (2005), made studies on post harvest loss of grapes by microbes. *T. harzianum* was found superior as compared to *T. viride* in inhibiting the growth of *A. niger* showing maximum 52% inhibition, *Bipolaris tetramera, Botryodiplodia theobromae* showing 75.5 and 61.1% growth inhibition respectively, whereas *T. viride* showed maximum 62.2% growth inhibition of *A. fumigatus*. *T. harzianum* was found effective in checking the growth of *A. niger, B. tetramera, B. theobromae* while *T. viride* was found superior against *A. fumigatus* as compared to *T. harzianum*.

Sharma et al., (2005) observed that *T. harzianum* exhibited maximum inhibition of *Aspergillus niger* and *Macrophomina phaseolina* and gave maximum germination of pea seeds, seedling vigor and minimum seedling mortality while *T. viride* showed maximum per cent germination of pea seeds against *Alternaria alternata, Fusarium oxysporum f. sp. pisi* and *Rhizoctonia solani*.

Shukla and Chaudhary (2005) studied the effects of volatile compounds of *Trichoderma*. They found that *Trichoderma harzianum* inhibited colony growth of *F. udum* by 24.2%, *Trichoderma viride* by 21.9% and *Trichoderma virens* by 17.1%; they were similar for their effect in reducing the conidia production (92.5-93.7%) against conidia production of *Fusarium udum*.

Upadhyay and Manjhi (2005) made studies on Pigeon pea seeds treated with spore suspension of *Trichoderma* (3 concentrations) and *Cajanus Rhizobium* (50 ml/kg seed) when placed on yeast extract manitol agar medium, reduction in growth of *T. viride* by 27.05 to 27.84% was observed in association with *Rhizobium*.

Sharma et al. (2005) isolated *Trichoderma* species from alkaline soils gave good degree of inhibition of pathogens of *Sclerotium rolfsii, Rhizoctonia solani*, for *Sclerotinia sclerotiorum* it was 77.39%. Also showed significantly higher root length (4.52 cm) and shoot length (4.70 cm) with 79.15% chilly seed germination.
Srivastava and Singh (2005) found that loose smut of wheat caused by *Ustilago segetum* var. *tritici* was controlled completely through seed treatment (by soaking seeds in culture filtrate of the bioagents for twelve hours) with the bioagents *Trichoderma viride*, *T. harzianum* and *T. koningii* in combination with Vitavax @ 0.125 g / kg seed during three years of trial. *Trichoderma harzianum* and *T. koningii* were compared with known bioagents viz., *Gliocladium virens*, *Trichoderma* sp. (Pantnagar isolate) and *Pseudomonas fluorescens* (Pantnagar isolate) by dual culture method, against pea wilt root rot complex pathogens namely *Fusarium solani* f. sp. *pisi*, *F. oxysporum* f. sp. *pisi*, *Rhizoctonia solani*, *Phoma medicaginis* var. *pinodella* and *Sclerotinia sclerotiorum* (Paul and Meena Devi, 2005). Both species of *Trichoderma* were effective against three pathogens. Hyphal interaction studies indicated several abnormalities in the hyphae of the pathogens viz., suppressed growth, excessive branching, excessive vacuolations, penetration, hyphal apex deformation, hyphal sliming, coiling and lyses. Population dynamics studies of the bioagents revealed that the population increase was maximum at 25°C, 30-35% soil moisture and pH.

Israel et al. (2005) studied influence of combined application of *Trichoderma* and Phorate on microbial population dynamics in sandy soils. The survival of *T. harzianum* was higher when added along with Phorate compared to its addition after 7 days of Phorate application in wet and dry soils. However, viable Propagule of *T. harzianum* decreased drastically at 2000-ppm concentration of Phorate in dry soil. These results suggest that *T. harzianum* can be combined with Phorate up to a certain concentration. Subsequently, combinations of treatments involving *T. harzianum* and Phorate were tested at farmer’s field in three arid districts of Rajasthan. The treatment involving bio agents and insecticide contributed towards regeneration of branches in a few of the affected trees. Variations in the microbial population and *T. harzianum* was estimated at 0-15 cm soil depth in the samples collected from the vicinity of the trunk of tree and at a distance of 2 cm in Churu district, no significant changes were recorded in counts of total actinomycetes but invariably total bacteria decreased after incorporation of these agents.