

Carbendazim Tolerant Mutant of *Gliocladium virens* for the Management of Damping off of Cabbage Caused by *Rhizoctonia solani* Kuehn

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Abstract: Nine stable mutants of *Gliocladium virens* have been developed by UV irradiation at wavelength of 260 nm for different exposure periods (viz., 60, 80 and 100 minutes). On the basis of higher growth parameters three of the best mutants (GvM1, GvM2 and GvM3) have been selected for further experiments. The wild strain is differed from mutant biotype in terms of colonial morphology, growth pattern, sporulation, antagonistic efficacy and fungicide tolerance. The mutant biotypes can tolerate higher concentration of carbendazim upto 50 ppm, while the wild strain fails to tolerate even 10 ppm concentration. Antagonistic potentiality of the mutants was tested against causal agent of damping off of cabbage (*Rhizoctonia solani*) in both in vitro and pot condition. Results were found to be more efficient for the mutants than the wild type in each parameter considered during the investigation viz. reduction in radial growth, percent inhibition of damping off, suppression of sclerotial growth and effect on growth parameters of the shoot and root length of cabbage seedlings.

Keywords: Mutants, *Gliocladium virens*, antagonistic, tolerance

1. Introduction

The cabbage crop is extremely susceptible to attack of the pathogen *Rhizoctonia solani* Kuehn (Teleomorph : *Corticium sasaki* (Shirali) Matumoto = *Thanetophorus cucumeris* (Frank Donk) which causes damping off, wirestem, bottom rot, head rot and root rot . Among these damping off is a serious problem, often leading to considerable mortality of cabbage seedlings by inducing pre and post emergence damping off (Singh, 1987). Due to the non specialized soil-borne nature the pathogen, it is very difficult to control the disease in the nursery bed. However the pathogen can be checked by seed treatment and soil drenching at frequent intervals with a safe fungicide (Tripathi, 2001), but none of the chemical is free from certain drawbacks such as higher cost, being easily available, development of resistant by pathogen, pollution hazards and harmful towards plants and animals. Due to high rainfall in north eastern region very often farmers fail to get desired results of the application of chemicals. As an alternative to the chemical control the antagonistic fungal isolates have been found to be effective for the management of damping-off of vegetables and other crops. However, most of them are very much sensitive to carbendazim which is often used as a seed-dressing fungicide to eliminate the seed-borne as well as soil borne fungal pathogens. In the present investigation an attempt has been made to overcome this limitation by genetic manipulation through the induction of mutants and thereby improving their biological control efficacy.

2. Materials and Methods

The pure culture of the wild type of the antagonist (*Gliocladium virens*) was collected from the culture collection of Department of Plant Pathology, Assam Agricultural University, Jorhat. The pathogen (*Rhizoctonia solani*) was isolated from the infected seedlings of cabbage showing typical symptoms of damping off, collected from the Horticultural orchard of Assam Agricultural University,

Jorhat. Both of the pure culture were maintained in Potato Dextrose Agar (PDA) medium and incubated at 28±1°C. The experiment was conducted in Assam Agricultural University, Jorhat during the rabi season of the year 2012-2013

Development of mutant of *G. virens* through UV-radiation

Conidial suspension *G. virens* was allowed to grow in PDA medium and plates were exposed to UV-radiation chamber at 260 nm maintaining a 29 cm distance from UV-tube for different time periods viz., 0, 60, 80 and 100 mins. After the irradiation, the plates were incubated at 25±1°C under alternate dark and light period. Colony developed were picked and grown on PDA followed by subsequent subculturing for thrice. Then the cycle of UV exposure was repeated again for once and colonies formed were isolated and grown on PDA by incubating at 25±1°C.

Induction of carbendazim tolerance in *G. virens*

The stable colonies developed after repeated UV irradiation were picked and grown in PDA amended with 2 ppm carbendazim (2 mg/lit of PDA) and 500 mg/lit streptomycin. The plates were incubated at 25±1°C and the colonies developed after 5 days were isolated, purified with serial dilution technique and subcultured thrice on PDA without carbendazim to test their stability. The stable colonies were grown on PDA amended with 5 ppm (5 mg/ lit of PDA) and streptomycin (500 mg/lit) and sub cultured 3-times on PDA without carbendazim to test their stability after next higher concentration. The same method was repeated for 10, 50 and 100 ppm carbendazim (10 mg/lit, 50 mg/lit and 100 mg/lit of PDA) and sub cultured on PDA to test their stability. The stable colonies developed were picked, transferred to PDA and incubated at 25±1°C.

The mutants showing higher degree of tolerance to carbendazim were grown on fungicide free PDA medium for studying their radial growth. The radial growth was recorded at 24 h interval upto 72 h. Then three stable mutants were

selected after 72 h of incubation on the basis of their higher radial growth and were designated as GvM1, GvM2, GvM3 and wild as Gv-Wild. The phenotypic and morphological characters of the mutants were studied after 72 h of incubation.

Inhibitory test of carbendazim tolerant UV irradiated mutant of *G. virens* against *R. solani* in vitro

The antagonistic potentiality of the wild and mutant *G. virens* were evaluated against *R. solani* in vitro by dual

culture method (Mukhopadhyay *et al.*, 1986). The dual culture assays were replicated six times for each treatment. The zones of inhibition and restriction in the radial growth of the pathogen observed were measured after 24 hrs of incubation till the complete coverage of control plates by the pathogen at an interval of 24 h. The statistical analysis for the data was done by one-way ANOVA. The per cent inhibition of growth of the pathogen was calculated by using the following formula

$$\text{Inhibition (\%)} = \frac{\text{Colony diameter in control} - \text{colony diameter in treatment}}{\text{Colony diameter in control}} \times 100$$

Antagonistic tests on the efficacy of wild and mutant *G. virens* on the production of sclerotia by *R. solani*

Ability of the wild and mutant *G. virens* for suppressing the sclerotia production of *R. Solani* was done by using MSM (4%). Each treatment was replicated seven times and observations on number of sclerotia produced in different treatments were recorded. Fresh weight and dry weight of sclerotia were also taken. The statistical analysis for the data was done by one-way ANOVA. The data were transformed to arcsine values before analysis.

Comparative study on growth and colonization by wild and mutant *G. virens* on sterilized soil

Thirty day old culture of *G. virens* (wild and mutant) grown on wheat bran @ 10 gm/kg of soil were applied on plastic pots containing sterilized soil. Each treatment was replicated for eight times and arranged in completely randomised block design. Observation on colonization of wild and mutant *G. virens* in the sterilized soil was taken at 0, 15 and 30 days after inoculation by taking one gram of soil from each pot and cfu was estimated with serial dilution technique using TSM.

Potentiality of mutant *G. virens* on reduction of damping off of cabbage

The pots were laid out in completely randomized block design (CRD) with three replication for each treatment. The soil for sterilized treatment was fumigated with formaline 40% before twenty five days of sowing. The soil at all the pots except the uninoculated control, was inoculated with 15 day old *R. solani* grown on MSM @ 2 gm/kg of soil. Cabbage seeds (cv. Pride of India) were treated with wild and mutant *G. virens* and chemical Bavistin. The treated seeds were sown @ 100 seeds/per pot.

3. Results

Induction of Carbendazim tolerant mutant of *Gliocladium virens*

On exposing the conidial suspension of wild *G. virens* to UV-radiation for different periods viz., 0, 60, 80 and 100 minutes and subsequent subculturing at different concentrations of carbendazim (viz., 0, 2, 5, 10, 50 and 100 ppm), except the wild, all the UV-irradiated *G. virens* were found to be capable of producing colonies upto 50 ppm of carbendazim (Table 1). It was also observed that irrespective of wild and all UV irradiated *G. virens*, could developed colonies upto 5 ppm of carbendazim. Moreover the wild *G. virens* was unable to develop any colony at 10 ppm concentration of fungicide while UV irradiated mutants of

G. virens could developed colonies at the same concentration after exposing to different period of time. A maximum of 8.6×10^5 cfu ml⁻¹ colonies of *G. virens* were recorded at 10 ppm concentration after exposing for 100 mins which was followed by 5.6×10^5 cfu ml⁻¹ and 2×10^5 cfu ml⁻¹ colonies at 10 ppm of carbendazim after exposing to 80 and 60 mins respectively (Table 1). When the concentration of the carbendazim was increased to 50 ppm, it was observed that there was no single colony developed in wild *G. virens*, while the UV irradiated *G. virens* developed stable colonies which was found to be less in numbers as compared to 10 ppm concentration. The maximum numbers of stable colonies 5.2×10^5 cfu/ml was observed in 100 mins irradiated *G. virens* which was followed by the 80 mins irradiated isolates (3.2×10^5 cfu/ml), 60 mins (1.0×10^5 cfu/ml) and wild type (0.0×10^5 cfu/ml). Similarly, none of the *G. virens* (both wild and mutant) could not produce any colony when the concentration was further increased to 100 ppm (Table 1). It was also noted that colony development of UV-irradiated *G. virens* were in decreasing trend with increasing concentrations of carbendazim i.e. the growth of stable colonies were inversely proportionate to the concentrations of carbendazim, which means higher was the concentration, lower the growth of stable colony. Only nine numbers of stable colonies were developed from the repeated exposure cycles of UV and carbendazim and they were designated as mutant GvR₆₀₋₁ (after 60 mins), GvR₈₀₋₁, GvR₈₀₋₂, GvR₈₀₋₃ (after 80 mins), GvR₁₀₀₋₁, GvR₁₀₀₋₂, GvR₁₀₀₋₃, GvR₁₀₀₋₄ and GvR₁₀₀₋₅ (after 100 mins). The best mutants were selected and designated from these nine stable mutants by culturing in media without carbendazim on the basis of their radial growth (mm) (Table 2). The radial growth of UV- irradiated *G. virens* at 100 mins showed significantly higher radial growth as compared to wild, 60 and 80 mins of UV-irradiation at all period of incubation (Table 2).

Three stable mutants were selected on the basis of their radial growth after 72 h of incubation from 9 numbers of stable colonies after 3rd generation. The maximum radial growth (87.66 mm) at 78 h was recorded in GvR₁₀₀₋₂. This was designated as GvM₁. This was followed by GvR₁₀₀₋₅ (74 mm) designated as GvM₂ and GvR₈₀₋₁ (67.68 mm) designated as GvM₃ and GvWild (55.67mm), respectively. Minimum growth was recorded at UV-irradiated GvR₈₀₋₃ at 80 mins (54 mm). All the other colonies showed significantly lesser colony diameter including wild *G. virens*. The phenotypic and morphological characters recorded after 72 h of incubation *G. virens* mutants are as given below.

Mutants	Phenotypic characteristics
Gv-Wild	Colony initially white later turn to light greenish to yellowish in colour with very light yellow pigmentation. Concentric rings are produced after 72 h of incubation.
GvM ₁ (GvR ₁₀₀₋₂)	Initially colony whitish, later turn greenish with yellow pigmentation.
GvM ₂ (GvR ₁₀₀₋₅)	Colony with whitish mycelia in the periphery, dark greenish in the centre. No prominent yellow pigmentation.
GvM ₃ (GvR ₈₀₋₁)	White colony, sporulate profusely and shows faster growth rate. Colony with heavy yellow pigmentation.

Antagonistic effect of carbendazim tolerant mutant of *G. virens* against *R.solani* in vitro

The *in vitro* antagonistic test showed a considerable differences between the three stable mutants and the wild *G. virens* in their ability to inhibit the radial growth of *R. solani* (Table 3). Irrespective of all the treatments the radial growth of *R. solani* was significantly reduced at all the time of incubation as compared to inoculated control. However, mutant GvM₁ (59.67%) was found to be the most effective in reducing the radial growth of the pathogen in all the periods after incubation which was followed by GvM₂ (51.93%). Both GvM₃ and Gv-Wild were less inhibitory at par on reduction of growth of the pathogen in comparison to other two (GvM₁ and GvM₂) in all hours of incubation.

Effect on the Sclerotial growth of *R. solani* in MSM 4% in presence of wild and mutant *G. virens*

Sclerotial development of *R. solani* was found to be arrested in all the treatments when *G. Virens* (Wild and mutant) was inoculated along with *R. solani*. However, maximum suppression was found when mutant GvM₂ was treated in MSM 4% with *R. solani*, which is followed by GvM₁, GvM₃ and Gv-Wild. The inhibitory effect on the development was represented by both in decreasing the number, fresh weight and dry weight of sclerotia (Table 4).

Growth and Colonization of wild and mutant *G. virens* on sterilized soil

During the observation period (upto 30 days) colony forming unit (cfu) was significantly higher for the mutant GvM₁ which was followed by GvM₃, GvM₂ and Gv-Wild. The population of both wild and mutant *G. virens* in the sterilized soil decreased with increase in storage period upto 30 days (Table 5). As the population of mutant GvM₁ in the sterilized soil is higher even in prolonged storage period therefore it is selected as the best mutant for further evaluation.

Potentiality of wild and mutant of *G. virens* on reduction of damping off of cabbage in pot condition.

All the seed treatments with mutant and wild *G. virens* were found to be effective in reducing pre and post emergence damping off. However maximum reduction of damping off was achieved when the seeds were treated with carbendazim @ 0.3% (87.79%), which was followed by the seed treatment with the best mutant (GvM₁) in both sterilized and unsterilized soil. Maximum disease incidence was found when soil application was done with *R. solani* alone (Table 6).

Effect of wild and mutant of *G. virens* on growth parameters of cabbage seedlings

Effect of wild and mutant of *G. virens* on growth and vigour of cabbage seedlings was evaluated under pot condition.

Shoot length (cm/plant)

Shoot length was increased in all the treatments with wild and mutant of *G. virens*. However, seed treatment with the mutant GvM₁ (in both sterilized and unsterilized soil) was more effective in increasing the shoot length, which was followed by seed treatment with Gv-wild. The seed treatment with carbendazim is also found to have positive effects in increasing shoot length and is ranked after the Gv-wild seed treatment. The lowest shoot length was observed at inoculated control.

Root length (cm/plant)

Seed treatment with mutant GvM₁ in both sterilised and unsterilized soil was found most effective in increasing root length followed by seed treatments with *G. virens* (wild). Seed treatment with carbendazim is found to be at par with the seed treatment with Gv-Wild in unsterilized soil. The lowest root length was recorded at inoculated control (Table 8).

4. Discussion

Induction of mutant of *Trichoderma* spp. by UV and Gamma irradiation for fungicide tolerance were reported by many earlier workers (Kumar *et al.*, 1999; Saikia *et al.*, 2000; Mech *et al.*, 2006; Roy *et al.*, 2005; Dutta *et al.*, 2008; Madhavi *et al.*, 2008). Silva and Melo (1994) tested *in vitro* tolerance of wild strains of *T. harzianum* and *T. viride* to different concentrations of benomyl in which they observed that the colony growth for all the strains were reduced to 50 per cent even at 2.5 ppm concentration of the fungicide. Saikia (2000) reported that conidial exposure of *T. viride* to UV-radiation induce mutant strains, which could tolerate upto 50 ppm carbendazim while the growth of wild type was completely inhibited at this concentration. Mech *et al.* (2006) found that mutants of *T. harzianum* obtained from prolong and repeated exposure to UV-radiation can grow even at 0.1% carbendazim concentration, while the wild strains fail to grow. Mukherjee *et al.* (1993) reported that gamma radiated mutants of *G. virens* were insensitive to carboxin and carbendazim upto 20µgml⁻¹ to 5 µgml⁻¹. No stable colony of both the wild and mutant type at carbendazim concentration of 100 ppm in our investigation might be due to the sensitivity of the antagonist to high concentration of fungicide. Fewer stable colonies observed in this study might be due to sub lethal (some individuals survive) or sub vital (some individuals survive) effect of mutation caused by UV-irradiation (Singh, 2000). The action of UV radiation on thymine dimmers, hydroxylation of U and C which may help the organism to tolerate carbendazim even at 50 ppm concentration as observed in this study. Therefore it may be assumed that, the three stable isolate GvM₁, GvM₂ and GvM₃ obtained after a series of UV-irradiation and carbendazim treatment were mutants of wild strain of *G. virens*.

The degree of inhibition for the radial growth of the pathogen varied from wild type to the mutant isolates which

must be due to the ability of the mutants for producing chitinases, cellulases and fungitoxic metabolites at relatively comparable amounts to that of the wild type without losing their essential antagonistic qualities as earlier reported by Jayaraj (2003). Higher production of Chitinase and β -1,3-glucanase and other antifungal substances by mutant isolates have been reported by Mukherjee *et al.* (1997), which enables them to utilize the substrate more efficiently than wild strain. It might also be due to the more antagonistic capacity and direct mycoparasitism potential of the mutants as compared to the wild (Viji *et al.*, 1993). The production of toxic metabolites including gliotoxin, gliovirin and fungal cell wall degrading enzymes like glucanases, chitinase, etc. are the main component of antagonism of *Gliocladium* (Roy *et al.* 2005). It was also experimentally proved that studied mutation can alter the enzyme producing ability in *G. virens* (Papavizas, Roberts and Kim, 1990). In their respective investigations they found a wide variation in β -1-3, glucanase, β -1, 4 glucanase, chitinase and cellulose activity, when wild and mutant isolates of *T. virens* were grown under both inducible and non-inducible condition. The synthesis of chitinase was found more in the mutant isolates of *G. virens* than their parental wild type which was clearly reflected in their greater *in vitro* antagonistic potential against the test pathogen. Therefore, it can be concluded that the mutant isolates developed through mutation might have the potentiality of increased extracellular mycolytic enzyme production and that increased production undoubtedly responsible for improved antagonistic potential over their wild types. The increased enzymatic enhancement may be due to altered increased endoplasmic reticulum content of the mutants (Ghosh *et al.*, 1982). The increased metabolism in the mutants of *T. virens* may be due to positive regulation of the cAMP signaling. An adenylate-cyclase-encoding gene, *tac1* is also believed to be involved in regulation of metabolism and antagonistic activity of *T. virens* which effect the growth, germination, mycoparasitism and secondary metabolism (Mukherjee *et al.*, 2007). UV induced mutation may not only affect the chitinase genes directly but also affect the synthesis and secretion of chitinase enzymes. Mendoza-mendoza *et al.* (2003) reported that deletion of *tvk1* gene from *T. virens* through UV-irradiation considerably increases biocontrol effectivity of the fungus. Hence, deletion of the respective genes causes increased mycoparasitic efficiency. The improvement in enzyme activity may be due to the photolysis of pyrimidines in adjacent pyrimidines to form dimers. They may cause error at the next replication and so result in mutation. The gene responsible for the production of cellulase may be over expressed due to mutation, which results in increase enzymatic activity in the mutant types (Chand *et al.*, 2005). Among the three mutant isolates developed here, the antagonistic effect of GvM₁ was more predominant which may be due to chromosomal mutation.

The suppression of the sclerotia production by wild and mutant *G. virens* must be due to direct suppressive effect on germination of sclerotia produced by *R. solani* (Mech *et al.* 2006). The greater efficacy showed by the mutant GvM₂ in reducing the sclerotial production of *R. solani*, may be due to inhibitory activity of metabolites that are harmful to the pathogen thereby reducing the primordia formation. The viability of whatever sclerotia have been produced was reduced due to the mycoparasitic action of the antagonist,

which utilizes the sclerotial cell contents for its own growth. The antagonistic fungal isolates utilize the sclerotial cell content and sporulate on sclerotial surface and inside the digested region resulting in degradation of the target sclerotia (Elad *et al.* 1981 and Chet *et al.* 1981).

The prolong survivability of the mutants in the sterilized soil may be due to formation of chlamydospores which show reduced germination both in unsterilized soil and on sterilized soil surface. The considerable difference between the cfu g⁻¹ soil recovered for each wild and mutant of *G. virens* after a regular interval may be due to the correlation with the temperature and other parameters. It may be concluded that the performance in the soil and the maintenance of their active propagule in the pot condition by the wild and the mutant *G. virens* strains tested in this work after storage at room temperature, not only contributes to the understanding of the behavior of these fungi in soil in laboratory condition, but it also help in having an idea about to provide the basis for a practical use of them at the field level.

The superiority of the mutant in reducing the pre and post emergence damping off is due to prevention of colonization by the pathogen in plant parts as compared to their parental wild type strain (*G. virens*). The result is in harmony with many successful attempts which have been carried out to improve the biocontrol abilities by mutagenic selection of different antagonistic fungal strains (Cassiolato *et al.*, 1997; Mukherjee *et al.*, 1999 and Mohamed and Haggag, 2002).

Potentiality of bioagent (wild and mutant of *G. virens*) in increased growth response and seedling vigour in terms of increased root length, shoot length and dry matter production is due to the production of some growth promoting substances. Similar observations were made by Windham *et al.* (1986) and Chang *et al.* (1986). Roy (1997) also reported that seed treatment with *Trichoderma* spp. enhanced seedling vigour along with increase in root and shoot length and dry weight of root and shoot of cabbage seedlings as the antagonist *Trichoderma* has the ability to produce growth promoting substances. Moreover, the increased growth response may be due to the ability of *Trichoderma* to inhibit minor pathogens in the rhizosphere which might induce seed rots and pre-emergence damping off. Some investigators reported that the increased growth response caused by *Trichoderma* isolates resulted in increased root area and root lengths may be related to the effect on root system. He also suggested a direct role for *T. harzianum* in mineral uptake by the plant at a very early stage of the fungal-plant association. In addition, Harman (2000) established that *Trichoderma* spp. are opportunistic plant colonizers that affect plant growth by promoting abundant and healthy plant roots, possibly via the production of plant hormones. Increased growth response has been demonstrated by several other investigators (Anusuya and Jayarajan, 1998).

References

- [1] Anusuya, D. and Jayarajan, R. (1998). Solubilization of phosphorus by *Trichoderma viride*. *Current Science*. 74: 464-466.

- [2] Cassiolato, A.M.R.; Baker, R. and De Melo, I.S. (1997). Effect of *Trichoderma harzianum* mutants on formation and carpogenic germination of *Sclerotinia sclerotiorum* sclerotia and lettuce plant survival. *Fitopatologia Brasileira*. **22**:34-38.
- [3] Chand, P.; Aruna, A.; Maqsood, A.M. and Rao, L.V. (2005). Novel mutation method for increased cellulase production. *J. App. Microbiol.* **98**(2): 318-323.
- [4] Chet, I.; Harman, G.E. and Baker, R. (1981). *Trichoderma hamatum* : Its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. *Microb. Ecol.* **7**: 29-38.
- [5] Dutta, P. (2008) Biological management of white mold of French bean by *Sclerotinia sclerotiorum* (Lib) de Bary by some local antagonists. Ph.D. thesis Assam Agric. University.
- [6] Elad, Y.; Chet, I. and Henis, Y. (1981). Biological control of *Rhizoctonia solani* in strawberry fields by *Trichoderma* spp. from soil. *Phytoparasitica* **9**: 59-67.
- [7] Ghosh, A.; Al-Rabaii, S. and Ghosh, B.K. (1982). Increased endoplasmic reticulum content of a mutant of *Trichoderma reesei* (RUT-C30) in relation to cellulase synthesis.
- [8] Harman, G. E. (2000). Myths and dogmas of biocontrol: Changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Pl. Dis.* **84**: 377-393.
- [9] Jayaraj, J. and Radhakrishnan, N.V. (2003). Development of UV induced carbendazim-resistant mutants of *Trichoderma harzianum* for integrated control of damping-off disease of cotton caused by *Rhizoctonia solani*. *J. Plant Dis. Protect.* **110**: 449-460.
- [10] Kumar, A. and Gupta, J.P. (1999). Variation in enzyme activity of tebuconazole tolerant biotypes of *Trichoderma viride*. *Indian Phytopath.* **53**: 263-266.
- [11] Madhavi, M.; Kumar, C.; Chandra, P.; Reddy, D.R. and Singh, T.V.K. (2008). Compatibility of mutant isolates of *Trichoderma* spp. with agrochemicals. *J. Biol. Control* **22**: 51-55.
- [12] Mech, S. (2006). UV-radiated mutant of *Trichoderma harzianum* Rifai and its carrier based formulation for management of white mold of French bean. M.Sc.(Agri.) Thesis, Assam Agric. Univ., Jorhat, Assam.
- [13] Mendoza, M.A.; Pozo, M.J.; Grzegorski, D.; Martinez, P.; Garcia, J.M.; Olmedo-Monfil, V.; Cortes, C.; Kenerley, C. and Herrera-Estrella, A. (2003). Enhanced biocontrol activity of *Trichoderma* through inactivation of a mitogen-activated protein kinase. *Proc. Natl. Acad. Sci. U.S.A.* **100**: 15965-15970.
- [14] Mohamed, H.A.A. and Haggag, W.M. (2002). Enhancement of antifungal metabolites production from gamma-ray induced mutants of some *Trichoderma* species for control onion white rot disease. *Pl. Path. Bull.* **11**: 45-56.
- [15] Mukherjee, P.K. and Mukhopadhyay, A.N. (1993). Induction of stable mutants of *Gliocladium virens* by gamma irradiation. *Indian Phytopath.* **46**: 393-397.
- [16] Mukherjee, P.K. and Tharayil, T.G. (2007). Induction of mutation in *Trichoderma virens* using UV-irradiation. *Recent trends in horticultural biotechnology*. Vol. II.
- [17] Mukherjee, P.K.; Haware, P.P. and Raghu, K. (1997). Induction and evaluation of benomyl – tolerant mutants of *Trichoderma viride* for biological control of *Botrytis* grey mold of chickpea. *Indian Phytopath.* **50**: 485-489.
- [18] Mukherjee, P.K.; Sherkhane, P.D. and Murthy, N.B.K. (1999). Induction of stable benomyl tolerant phenotypic mutants of *Trichoderma pseudokoningii* MTCC3011, and their evaluation for antagonistic and biocontrol potential. *Indian J. Expt. Biol.* **37**: 710-712.
- [19] Mukhopadhyaya, A.N. and Chandra, I. (1986). Biological control of sugarbeet and tobacco damping-off by *Trichoderma harzianum*. II. *Conf. on Plant Prot. In The Tropics, Malaysia*, pp. 60-65.
- [20] Papavizas, G.C.; Roberts, D.P. and KIM, K.K. (1990). Development of mutants of *Gliocladium virens* tolerant to benomyl. *Canadian J. Microbiol.* **36**: 484-489.
- [21] Roy, M.; Jash, S.; Khalko, S.; Bose, S.; and Pan, S. (2005). Morphological and physiological characterization of some mutant isolates of *Gliocladium virens*, a potential mycoparasite of sclerotial plant pathogens, Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia - 741 252, India.
- [22] Roy, S.K. (1997). Biological seed treatment with *Trichoderma* species for management of damping-off of cabbage caused by *Rhizoctonia solani* Kuehn. M.Sc.(Agri.) Thesis, AAU, Jorhat-13.
- [23] Saikia, M.K. (2000). Integrated management of stem rot of cauliflower caused by *Rhizoctonia solani* Kuhn. Ph.D. Thesis submitted to C.C.S. Agric.Univ. Hisar, Haryana. India.
- [24] Silva, A.C.F and Melo, I.S. (1994) Reduction of *Trichoderma harzianum* Rifai and *Trichoderma viride* Pers. Ex. Fr. Grey isolates to benomyl and iprodione *in vitro*. *Revista de Agricultura.* **69**: 231-299.
- [25] Singh, B.D. (2000). Mutation. In A text book of plant breeding. Kalyani Publishers, pp. 165-180.
- [26] Singh, R.S. (1987). *Diseases of Vegetable Crops*. 2nd Edn., Oxford and IBH Publ. Col. Pvt. Ltd., New Delhi, p. 143.
- [27] Tripathi, N.N. and Anitha, K. (2001). Screening of fungicides against seedling mortality to okra caused by *Rhizoctonia solani* and *Pythium aphanidermatum*. *Plant Dis. Res.* **16**: 52-56.
- [28] Viji, G.; Baby, U. I. and Rao, M. K. (1993). Induction of fungicidal resistance in *Trichoderma* spp. through UV irradiation. *Indian J. Microbiol.* **33**: 125-129.
- [29] Windham, M.T.; Elad, Y. and Baker, R. (1986). A mechanism for increased plant growth induced by *Trichoderma* spp. *Phytopath.* **6**: 518-521.

Table 1: Growth of *Gliocladium virens* ($\times 10^5$ cfu/ml) in PDA amended with carbendazim at different concentrations after exposing to UV-irradiation

Exposure of <i>G. virens</i> to UV-irradiation for different times (minutes)	Population of <i>G. virens</i> ($\times 10^5$ cfu/ml) in PDA amended with carbendazim at different concentration after exposing to UV-irradiation					
	0 ppm	2 ppm	5 ppm	10 ppm	50 ppm	100 ppm

0	51.6	18.6 ^c	4.0 ^d	0.0 ^d	0.0 ^d	0
60	47.4	27.0 ^b	8.4 ^c	2.0 ^c	1.0 ^c	0
80	53.2	30.8 ^a	12.6 ^b	5.6 ^b	3.2 ^b	0
100	54.0	32.8 ^a	15.8 ^a	8.6 ^a	5.2 ^a	0
S.Ed (±)	NS	1.08	0.72	0.64	0.59	-
CD _{0.05}	NS	2.29	1.54	1.36	1.25	-

Means within columns separated by Duncan's multiple range test P = 0.05

Figures followed by same letters are statistically not different

Table 2: Radial growth of UV-irradiated *Gliocladium virens* on PDA medium at different time of incubation

UV-irradiated <i>G. virens</i> isolates	Colony diameter of <i>G. virens</i> (mm) after different hours of incubation			Selected mutants
	24h	48h	72h	
GvR ₆₀₋₁	7 ^f	34 ^{de}	56 ^d	
GvR ₈₀₋₁	11.66 ^{bc}	36.67 ^d	67.67 ^c	GvM ₃
GvR ₈₀₋₂	9.33 ^{de}	25.67 ^e	55.67 ^d	
GvR ₈₀₋₃	8.33 ^{def}	29 ^f	54 ^d	
GvR ₁₀₀₋₁	11.33 ^c	34 ^{de}	56.33 ^d	
GvR ₁₀₀₋₂	15.46 ^a	56 ^a	87.66 ^a	GvM ₁
GvR ₁₀₀₋₃	13.33 ^a	44 ^c	56 ^d	
GvR ₁₀₀₋₄	7.66 ^{cd}	32.33 ^f	57 ^d	
GvR ₁₀₀₋₅	15.33 ^{ab}	49.33 ^b	74 ^b	GvM ₂
Gv Wild	10 ^{cd}	29.67 ^f	55.67 ^d	
S.Ed(±)	0.99	1.49	1.46	
CD _{0.05}	1.91	3.12	3.04	

Means within columns separated by Duncan's multiple range test P = 0.05 Figures followed by same letters are statistically not different

Table 3: Effect on radial growth and percent inhibition of *Rhizoctonia solani* by wild and mutant of *Gliocladium virens* in dual culture

Treatments	Radial growth (mm) at different hours of incubation			% inhibition at different hours of incubation		
	24 h.	48 h.	72 h.	24h.	48 h.	72 h.
T ₁ = <i>G. virens</i> (wild) + <i>R. solani</i>	21.22 ^b	42.47 ^b	56.82 ^b	54.44 (36.29) ^d	40.61 (29.59) ^d	36.87 (31.64) ^d
T ₂ = GvM ₁ + <i>R. solani</i>	10.37 ^e	23.47 ^e	36.3 ^e	77.78 (56.41) ^a	67.19 (49.86) ^a	59.67 (48.24) ^a
T ₃ = GvM ₂ + <i>R. solani</i>	16.23 ^d	32.28 ^d	43.27 ^d	65.18 (44.83) ^b	54.85 (40.05) ^b	51.93 (42.53) ^b
T ₄ = GvM ₃ + <i>R. solani</i>	19.13 ^c	37.78 ^c	50.13 ^c	58.92 (39.75) ^c	47.15 (34.33) ^c	44.29 (37.01) ^c
T ₅ = Control	46.63 ^a	71.57 ^a	90 ^a	-	-	-
S.Ed(±)	0.52	2.12	0.79	0.93	1.03	0.72
CD _{0.05}	1.08	4.37	1.62	1.9	2.14	1.49

Means within columns separated by Duncan's multiple range test P = 0.05

Figures followed by same letters are statistically not different

Figures in the parentheses are angular transformation value

Table 4: Sclerotial growth of *Rhizoctonia solani* in 4% MSM in presence of wild and mutant of *Gliocladium virens* in vitro

Treatments	Number of sclerotia	Fresh weight (g)	Dry weight (g)
T ₁ = <i>R. solani</i> + MSM	73.57 ^a	5.60 ^a	2.91 ^a
T ₂ = <i>G. virens</i> (wild) + <i>R. solani</i>	27.57 ^b	2.95 ^b	1.51 ^b
T ₃ = GvM ₁ + <i>R. solani</i>	16.14 ^c	1.51 ^c	0.77 ^c
T ₄ = GvM ₂ + <i>R. solani</i>	10.85 ^d	1.21 ^d	0.48 ^d
T ₅ = GvM ₃ + <i>R. solani</i>	10.42 ^d	1.09 ^d	0.47 ^d
S.Ed(±)	1.51	0.17	0.11
CD _{0.05}	3.09	0.34	0.21

Means within columns separated by Duncan's multiple range test P = 0.05
 Figures followed by same letters are statistically not different

Table 5: Growth and colonization of wild and mutant of *Gliocladium virens* (×10⁵ cfu/ml) on sterilized soil

Treatments	Population of wild and mutant of <i>G. virens</i> (×10 ⁵ cfu/ml) on sterilized soil		
	0 Days after inoculation	15 Days after inoculation	30 Days after inoculation
T ₁ = Sterilized soil + Wild <i>G. virens</i>	82.12 (1.91) ^d	18.87 (1.27) ^c	4.37 (0.61) ^c
T ₂ = Sterilized soil + GvM ₁	184.50 (2.26) ^a	58.62 (1.78) ^a	29.37 (1.47) ^a
T ₃ = Sterilized soil + GvM ₂	97.62 (1.98) ^c	39.12 (1.59) ^b	16.25 (1.21) ^b
T ₄ = Sterilized soil + GvM ₃	111.87 (2.04) ^b	34.50 (1.54) ^b	14.37 (1.15) ^b
S.Ed(±)	0.02	0.02	0.05
CD _{0.05}	0.03	0.05	0.11

Means within columns separated by Duncan's multiple range test P = 0.05
 Figures followed by same letters are statistically not different
 Figures within parentheses are log transformed values

Table 6: Effect of wild and mutant of *Gliocladium virens* on pre-emergence and post-emergence damping-off of cabbage in pot

Treatments	Pre-emergence damping-off (%)	Per cent disease control over inoculated control	Post-emergence damping-off (%)	Per cent disease control over inoculated control	Per cent disease incidence over inoculated control
T ₁ = Seed treatment with wild <i>G. virens</i> + <i>R. solani</i> + Sterilized soil	16.83 (24.21) ^c	71.12	8.94 (17.37) ^c	76.73	25.77
T ₂ = Seed treatment with mutant of <i>G. virens</i> + <i>R. solani</i> + sterilized soil	10.89 (19.22) ^d	81.28	5.79 (13.92) ^d	84.95	16.68
T ₃ = Seed treatment with wild <i>G. virens</i> + <i>R. solani</i> + Unsterilized soil	24.09 (29.39) ^b	58.67	10.52 (18.92) ^c	72.59	34.61
T ₄ = Seed treatment with mutant <i>G. virens</i> + <i>R. solani</i> + Unsterilized soil	17.10 (24.42) ^c	70.67	6.30 (14.53) ^d	83.52	23.41
T ₅ = Seed treatment with Carbendazim @ 0.3% + <i>R. solani</i>	8.68 (17.13) ^e	85.11	4.69 (12.50) ^b	87.79	13.37
T ₆ = Untreated seed + <i>R. solani</i> (Inoculated control)	58.28 (49.77) ^a	-	38.43 (38.31) ^a	-	96.72
T ₇ = Absolute control (Uninoculated control)	3.64 (10.85) ^f	93.75	1.58 (7.12) ^e	95.92	5.22
S.Ed(±)	1.0	-	0.76	-	-
CD _{0.05}	2.08	-	1.58	-	-

Means within columns separated by Duncan's multiple range test P = 0.05
 Figures followed by same letters are statistically not different
 Figures within parentheses are angular transformed values.

Table 7: Effect of wild and mutant of *Gliocladium virens* on shoot length and root length of cabbage seedlings in pot

Treatments	Shoot length (cm)	Per cent increase in shoot length	Root length (cm)	Per cent increase in root length
Seed treatment with wild <i>G. virens</i> + <i>R. solani</i> + Sterilized soil	4.67 ^c	50.43	2.97 ^c	34.78
Seed treatment with best mutant of <i>G. virens</i> + <i>R. solani</i> + sterilized soil	5.67 ^a	59.13	5.74 ^a	56.57
Seed treatment with wild <i>G. virens</i> + <i>R. solani</i> + Unsterilized soil	4.38 ^d	47.07	2.67 ^d	29.58
Seed treatment with mutant <i>G. virens</i> + <i>R. solani</i> + Unsterilized soil	4.88 ^b	52.56	4.35 ^b	49.15
Seed treatment with Carbendazim @ 0.3% + <i>R. solani</i>	3.43 ^e	32.40	2.7 ^d	30.32
Untreated seed + <i>R. solani</i> (Inoculated control)	2.32 ^g	-	1.68 ^f	-
Absolute control (Uninoculated control)	2.71 ^f	-	2.33 ^e	-

(±)	0.03	-	0.09	-
5	0.08	-	0.19	-

Means within columns separated by Duncan's multiple range test $P = 0.05$
Figures followed by same letters are statistically not different

