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Colonization of *Gliocladium virens* on Biologically Treated Chickpea Seeds for Biocontrol of Chickpea Wilt Complex

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Chickpea *(Cicer arietinum)* seeds treated with powdered preparation of *Gliocladium virens* (Gv) alone @ 3 g/kg seed or in combination with vitavax (0.1%) showed colonization of *G. virens* on seed coat, collar region, plumule and radicle. Microscopic examination revealed that colonization of seed with mycelia and spores of antagonist started 24 h after incubation. Major portion of the seedling was covered with in 48 hrs. Population dynamics of *G. virens* monitored at different time intervals in spermosphere, rhizosphere and non-rhizosphere region in pathogen infested and non-infested soil using *Trichoderma* Selective Medium showed that population of *G. virens* increased initially up to 30 days and then gradually declined. The highest population was observed in spermosphere (7×10^5/g) followed by rhizosphere (6.3×10^4/g), when seeds treated with Gv + vitavax were sown in pathogen infested soil.

Keywords: Chickpea, *Gliocladium virens,* colonization, population dynamics.

In order to protect the germinating seeds, seedlings and roots from pathogen infection, the biofungicides need to be delivered in a manner that allows the antagonist to rapidly colonize the seed coat, spermosphere and the developing rhizosphere at a density that is high enough to suppress the pathogen. The soil samples taken from Agricultural regions revealed that the natural populations of *Trichoderma* is rather low (Chet, 1987), which is not sufficient to suppress a pathogen. Therefore, there is a need of higher population of antagonist in the soil to suppress the pathogen. This could only be achieved by introducing the pathogen into the soil either directly or through seed treatment.

Biological seed treatment is potentially a very efficient means of applying biological control agents to soil, as it is a non-polluting delivery system and only a small amount of material is applied per hectare and in immediate contact with the target site (Taylor and Harman, 1990; Mukhopadhyay et al., 1992). Biological seed treatment has tremendous potential to make biological control success especially for seed and seedling diseases in vegetables, fruits, forest and other plantation crop nurseries (Mukhopadhyay, 1996). Seed treatment with fast growing fungal antagonist prevents seed decay and seedling blight by pre-emptying leaked materials and thus restricting prepenetrations and increase of pathogen biomas by providing a protective covering, producing pathogen inhibiting enzymes, antibiotics and occasionally by parasitism (Baker, 1987). The present

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paper highlights results on biological seed treatment of chickpea seeds with bioformulation of *Gliocladium virens* and its successful colonization with respect to control of chickpea wilt complex.

Materials and Methods

The antagonist *Gliocladium virens* Miller, Giddens, and Foster, isolate-1 (IMI No. 304061) from Biocontrol Laboratory, Department of Plant Pathology, GBPUA and T, Pantnagar was selected for the present investigations. The antagonist was multiplied on sorghum grains and colonized grains were air dried, powdered and sieved through 80 mesh sieve. The concentration of spore powder was standardized with the help of haemocytometer.

Colonization of G. virens *on chickpea seeds*

An experiment was conducted by treating the seeds with the powdered preparation of *G. virens* alone @ 3 g/kg seed or in combination with vitavax (0.1%), to assess the ability of *G. virens* to colonize the seed coat, collar region, plumule and root zone of germinating chickpea seeds. In first experiment the treated seeds were directly placed in Petri plates lined with moist blotter paper. In the second experiment the treated seeds were first sown in natural soil, uprooted after germination and then placed in Petri plates lined with moist blotter paper. The plates were incubated at 27 + 1 ºC. Seeds without any treatment served as check.

Light microscopy

Observations on colonization of *G. virens* on seed surface, collar region, plumule and radicle were made periodically under steriobinocular microscope.

Root printing

Treated seeds sown in natural soil were uprooted at seedling stage, 10 days after sowing (DAS). The extra soil was removed from the root portion. Root print was made by placing the root on modified Trichoderma Selective Medium (TSM) (Shrestha, 1992). Printed plates were incubated for four days at 27 + 1 ºC with 12 h light and 12 h dark conditions alternatively. The growth of antagonist on printed plates was observed under steriobinocular microscope.

Population dynamics

The experiment was conducted to find out the population dynamics of the antagonist in the spermosphere, rhizosphere and in non-rhizosphere regions of growing chickpea plants in pathogen infested soil. The pathogens, *Sclerotium rolfsii, Rhizoctonia solani, Fusarium solani* and *F. oxysporum* f. sp. *ciceri* associated with wilt complex were inoculated in 6" plastic pots filled with natural soil @ 0.5 g, 1.5 g, 6.0 g and 6.0 g, respec-

tively. The uninoculated natural soil (non-infested soil) filled in pots was also kept as a check. Chickpea seeds treated with powdered preparation of *G. virens* @ 3 g/kg seed alone or in combination with vitavax (0.1%) were sown in pathogen infested as well as in non-infested soil, taken in pots with three replications. Seeds without any treatment served as check. Soil surrounding the seed (spermosphere), the roots (rhizosphere) and from the pots up to a depth of 5 cm (non-rhizosphere) collected periodically at 15, 30, 45 and 60 DAS were air dried and ground to fine powder.

The soil samples were analyzed by soil dilution plate method to find out the colony forming units (CFU). One gram soil taken from each thoroughly mixed sample was suspended separately in 10 ml of sterilized distilled water. Further dilutions were made by transferring one ml of this suspension to nine ml of sterilized distilled water till the final dilution of 10^{-3} (rhizosphere and non-rhizosphere) and 10^{-4} (spermosphere) was obtained. One ml of this suspension was poured in modified TSM plates and spread evenly by horizontal tilting. The plates were incubated at 27 + 1 ºC for four days. Observations on number of cfu were recorded with an aid of colony counter.

Results and Discussion

Light microscopy

Treated seeds directly placed in moist Petri dishes observed under steriobinocular microscope showed that growth of *G. virens* on seed surface started 24 h after incubation. Major portion of the seed was colonized with the mycelia and spores of antagonist after 48 h of incubation. Colonization of antagonist on collar region, plumule and radicle was observed after four days of incubation. As far as colonization was concerned, no differences were observed in seeds treated with powdered bioformulation of *G. virens* alone or in combination with vitavax. No growth of antagonist was observed on untreated seeds.

Treated seeds sown in natural soil and thereafter transferred in moist Petri dishes after germination was also observed under steriobinocular microscope. Growth of antagonist on seed surface started after 12 h of incubation. Major portion of the seed, collar region and radicle were colonized by profuse growth of mycelia and spores on the seeds, treated with antagonist in combination with vitavax whereas, only a substantial portion of the seed was colonized with spores and mycelia of antagonist after 48 h of incubation on seeds treated with powdered preparation of *G. virens* alone *(Fig. 1).* The untreated seeds were completely colonized by undesirable fungi viz., *Penicillium, Rhizopus, Fusarium, Rhizoctonia* and *Sclerotium.*

These studies revealed that the chemical (vitavax) to which *G. virens* was insensitive, reduced the growth of other saprophytic fungi found in soil, thereby reducing the competition for the food and space which facilitated the antagonist to become the first colonizer at initial growth stage resulting in better proliferation of *G. virens* on seed parts. Harman et al. (1980) observed that *Trichoderma hamatum* colonized the radish seed coat when incubated for 2 days after seed treatment.

Fig. 1. Colonization of *Gliocladium virens* on chickpea seed. Conidiophores with conidial balls on seed coat

Root print

Mycelial colonization of *G. virens* was observed on root prints of treated chickpea seeds on TSM plates after 4 days of incubation whereas no colonization was observed in root prints of untreated seeds. This experiment showed that *G. virens* colonized the rhizoplane of chickpea seedlings *(Fig. 2).* Colonization of atagonistic microorganism on root was observed by Nemec et al. (1996) in capsicum, tomato and citrus, Kurakov and Kostina (1997) observed root colonization in tomatoes, cucumber, onions, barley and blue millet.

Population dynamics of G. virens

Since the natural population of a soil is rather low, usually not exceeding 10^2 cfu/g (Chet, 1987), which is insufficient to suppress the pathogens, the quantitative estimation of the introduced antagonist in the soil through seeds at regular time intervals is necessary to ascertain its establishment in the spermosphere, rhizosphere and non-rhizosphere

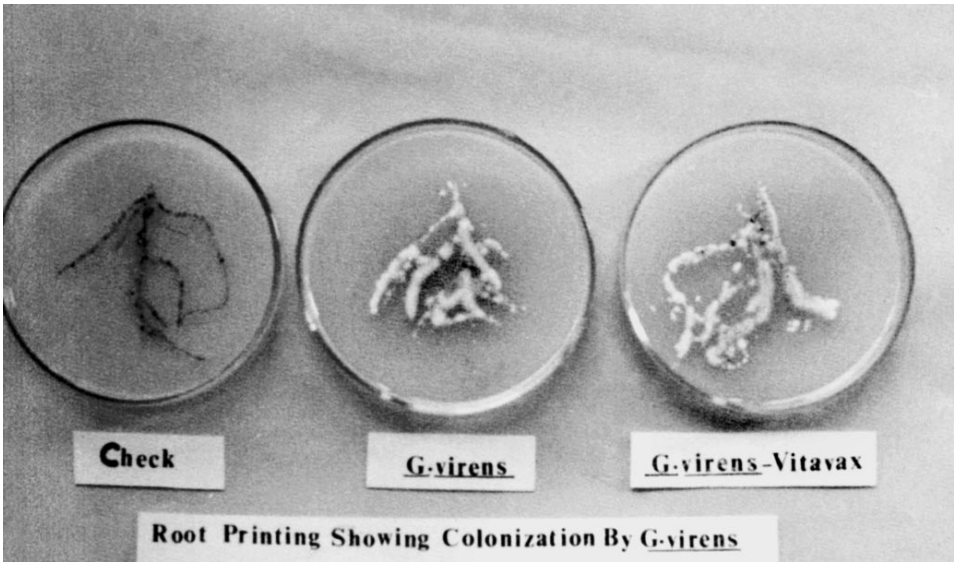

Fig. 2. Colonisation of *Gliocladium virens* on root of chickpea

regions. Implication of rhizosphere competence of the antagonist in the soil through seeds has been emphasized by various workers (Chao et al., 1986; Ahmad and Baker, 1988; Kumar and Marimuthu, 1997).

Results of present investigations have shown that the population of *G. virens* increased in first 30 days and sharply declined after 45 and 60 days of planting in all the regions i.e. spermosphere, rhizosphere and non-rhizosphere in pathogen infested as well as in non-infested soil when chickpea seeds were treated with *G. virens* alone or in combination with vitavax. The population of *G. virens* in check increased slightly up to 45 DAS in pathogen infested soil where as it decreased in non-infested soil. Gradual decline in the population level of the introduced antagonist *(Trichoderma/Gliocladium)* with advancing time has been reported by several workers (Papavizas, 1982; Lewis and Papavizas, 1984; Park et al., 1992).

The higher population of *G. virens* was observed in spermosphere (7×10^5) followed by rhizosphere (6.3×10^4) and non-rhizosphere (2.4×10^4) in pathogen infested soil as compared to non-infested soil in which it was 3.6×10^5 (spermosphere), 2.5×10^4 (rhizosphere) and 2.3×10^4 (non-rhizosphere) after 30 DAS in the combined treatment of *G. virens* and vitavax. However, seeds treated with *G. virens* alone showed the population 5.9×10^5 (spermosphere), 4.0×10^4 (rhizosphere) and 2.0×10^4 (non-rhizosphere) in pathogen infested soil *(Table 1)* as compared to 3.1×10^5 (spermosphere), 2.4×10^4 (rhizosphere) and 2.2×10^4 (non-rhizosphere) in non-infested soil *(Table 2).* The increased population of *G. virens* observed in treatment, Gv + vitavax, could be due to the fact that the vitavax provided primary protection to the seed from infection by other soil borne fungi. The

Table 1

Population dynamics of *G. virens* in pathogen infested soil

Treatment	Spermosphere (× 10^4 CFU/g soil)				Rhizosphere (× 10^3 CFU/g soil)				Non-rhizosphere (× 10^3 CFU/g soil)			
	Days after sowing											
	15	30	45	60	15	30	45	60	15	30	45	60
Gv	30.0	59.0	25.3	15.7	25.0	40.0	15.3	10.0	17.3	24.7	11.7	7.0
Gv+vitavax	34.0	70.0	30.7	19.7	26.7	63.3	17.3	11.3	20.3	25.0	14.7	9.7
Check	7.3	12.3	14.3	7.0	5.0	7.7	6.3	3.7	5.7	7.7	5.0	3.3
C. D. at 5%	9.0	11.4	9.5	8.0	10.8	16.7	10.9	4.1	5.9	6.8	5.5	4.3

antagonist first utilized the seed exudates as food base then proliferated on seed, utilized exudates in the spermosphere and subsequently in the rhizosphere and then spreading into the soil by multiplication. It was observed throughout the course of investigation that the soil infested with pathogens sustained more population of *G. virens* than in non-infested soil. The possible explanation may be that the antagonist parasitized or killed the pathogen and utilized them as a food base for further proliferation and multiplication. Harman et al. (1980) reported that *G. virens* parasitized the mycelia and sclerotia of the pathogen utilized as food base and proliferated into the soil. A higher *Trichoderma* population was observed in soil suppressive to plant pathogens (Chet and Baker, 1981; Aziz et al., 1997).

It can be concluded from the results of present investigations that chickpea seeds treated with powdered preparation of *G. virens* (3 g/kg seed) in integration with vitavax (0.1%) increased the efficacy of antagonist to colonize the seed coat, collar region and radicle of germinating seeds and spermosphere, rhizosphere and non-rhizosphere of growing chickpea plants, which helped in successful biological control of chickpea wilt complex.

Table 2

Population dynamics of *G. virens* in non-infested soil

Treatment	Spermosphere (× 10^4 CFU/g soil)				Rhizosphere (× 10^3 CFU/g soil)				Non-rhizosphere (× 10^3 CFU/g soil)			
	Days after sowing											
	15	30	45	60	15	30	45	60	15	30	45	60
Gv	18.7	31.0	15.0	8.3	17.3	24.7	11.7	7.0	17.0	22.0	12.7	10.3
Gv+vitavax	21.3	36.3	17.0	10.7	20.3	25.0	14.7	9.7	18.0	23.3	13.3	10.7
Check	2.7	5.7	11.7	4.0	7.7	5.0	5.7	3.3	5.0	4.7	3.3	2.0
C. D. at 5%	4.7	5.8	5.6	5.5	5.9	6.8	5.5	4.3	8.9	10.8	4.8	5.1

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