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Research Article

EFFECTS OF GLUCOSE BASE FORMULATION OF *TRICHODERMA VIRIDE* ON SEED GERMINATION AND SEEDLING PARAMETERS OF TOMATO (*SOLANUM LYCOPERSICUM*)

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ABSTRACT

Seed germination is the critical stage in the life cycle of crop plants. Environmental factors such as light, temperature, nutrient and water can regulate the seed germination. In the present study, influence of glucose along with the antagonistic fungus *Trichoderma viride* was tested for tomato seed germination in a complete randomized block design. Seed germination percentage, root length, shoot length and seedling vigour index were significantly improved by the application of glucose formulation of *T.viride.* The highest seed germination (92.66%) was recorded in the glucose formulation of *T.viride* (10g/kg of seed) which was significantly superior as compared to chemical fungicide thiram (89.33%), talc base (82.33%) and liquid base formulation (76.33%) of *T.viride.* The study indicates that glucose formulation of *T.viride* contributes and coordinately involved in the seed germination and seems to be promising strategy to improve the germination of tomato seeds.

Keywords: *Seed Germination, Glucose, Growth Promotion, Solanum lycopersicum*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop grown worldwide after potato (Olaniyi *et al*, 2010). Tomato seeds affected by many biotic and abiotic factors and causes delayed or erratic seed germination. Soil borne fungal diseases play a vital role in reducing of seed germination. Delayed and erratic germination create problems with fertilizer utilization, post emergence weed control, and uniform harvesting (Standifer, Wilson and Drummond, 1989). Seed germination is regulated in most of the plant species by two antagonistic plant hormones, ABA (Abscisic acid) and gibberellins. ABA is involved in the induction and maintenance of seed dormancy and inhibits seed germination (Nambara *et al*, 2010) whereas gibberellin promotes seed germination (Yamaguchi, 2008) and *T. viride* is known to produce gibberellin (Asaduzzaman , 2010) and auxins (Lalitha, Srujana and Arunalakshmi, 2012). *Trichoderma* also solubilized the phosphates, micro-nutrient and minerals such as Fe, Mn and Mg that have important role in plant growth (Altomare *et al*, 1999) along with secretion of exogenous enzymes, siderophores (Jalal, Love and Vander-Helm, 1987) and vitamins (Inbar, 1994; Kleifeld and Chet, 1992). It also control major and minor root infesting pathogens (Harman *et al*, 2004) in rhizosphere.

In the present study, antagonistic fungus *Trichoderma viride* was isolated from the soil, identified and cultivated for mass production. After incubation, harvested the spore aseptically and prepared its soluble powder formulation using glucose (dextrose) as a carrier. Then stability of formulation was studied along with effect of formulation on germination of tomato seeds. A number of formulations of *Trichoderma* *viride* are available in the market but yet they have certain lacunas like most of them are not completely soluble in water, high pH range, less shelf life, a problem of contamination etc. In the present attempt, with the support of DSIR (Department of Scientific and Industrial Research, Government of India) we developed glucose base formulation of *Trichoderma viride* which has extraordinary two years shelf life, high spore count, neutral pH range, complete water solubility and formulation is most suitable for seed treatment, drenching, drip, and foliar spray application. The highest percentage of germination of tomato seeds was recorded when this formulation tested through seed treatment which was significantly superior as compared to powder and liquid formulations of same strain of *T.viride.*

MATERIALS AND METHODS

Isolation of Trichoderma viride

Collected rhizosphere soil samples in the clean polybag from the field of tomato, stored them at 4^0C until used. Ten-gram soil was taken in 90 ml sterilized distilled water, mixed uniformly. Five-fold serial dilution of each soil sample prepared and 0.1 ml of last two dilutions plated on the selective medium of *Trichoderma* (Elad, Chet and Henis, 1982). Then plates were incubated at 28 ± 2^0C for 96 hours. After incubation individual colonies were isolated on fresh potato dextrose agar plates. Various morphological characteristics were observed for identification includes; colony appearance, growth pattern on Potato Dextrose Agar (PDA) and Cornmeal Dextrose Agar (CMD), the shapes and sizes of conidia, the branching patterns of conidiophores and phialides, the production of chlamydospores etc (Bissett, 1991, Rifai, 1969 and Samuels, 1996). The most promising isolate of *Trichoderma viride* was selected for mass multiplication (Bissett, 1991).

Mass multiplication of Trichoderma viride and formulation development

Mass multiplication of *Trichoderma viride* carried out on substrate sorghum grains. Surface sterilization of substrate (sorghum grains) was made for the production of contamination-free conidia using chlorine water, followed by washing with RO water. After washing grains were filled in poly bags for autoclaving at 121^0 C for twenty minutes (Patil *et al*, 2017). For the production of contamination-free conidia of *Trichoderma viride*, forty eight hour old mycelium broth of *Trichoderma* inoculated into each poly bags and bags were incubated at 28^0 C for five days for maximum sporulation. After sporulation, conidia of *Trichoderma* were harvested aseptically from sorghum grains, confirmed its purity and glucose, talcum, and liquid formulations were prepared using same amount of spore. After confirmation of purity and cfu count these formulations were tested for germination of tomato seeds.

Bio-Efficacy Testing

A replicated trial was conducted in a randomized block designed with seven treatments to evaluate the effects of formulations on seed germination. The seeds of tomato treated with the *T.viride* formulations (Table 1) were sown on the raised beds which were prepared using sterilized soil. The observations of seed germination were recorded after ten days of sowing and root length, shoot length and seedling vigour index (SVI) were recorded after thirty-five days of sowing. The tomato seedlings were critically and frequently observed for the manifestation of the phytotoxic effect and abnormalities due to formulations of *T. viride*.

RESULTS AND DISCUSSION

Effect on growth parameters

The perusal of the data (Table 1) revealed that all the treatments exhibited significantly higher seed germination, root length, shoot length and seedling vigour index (SVI) over untreated control. However, the seed treatment with glucose based *T.viride* at 10g/kg seed (T5) recorded significantly the highest seed germination (92.66%), root length (7.97cm), shoot length (14.90cm) and SVI (2097.91). This was followed by the seed treatment (T6) with the fungicide thiram at 2g/kg seed (89.33 %, 6.97cm, 13.95cm and 1869.79) and seed treatment (T1) with talc-based *T. viride* at 10g/kg seed (82.33%, 5.66cm, 11.80cm and 1598.21) with seed germination, root length, shoot length and SVI, respectively. Rest of the treatments also recorded maximum seed germination (70.33 to 76.33%), root length (4.22cm to 5.44cm), shoot length (8.43cm to 9.88cm) and SVI (882.81 to 1169.30), as against significantly least seed germination (56.33%), root length (3.59cm), shoot length (6.11cm), and SVI (546.62) in untreated control.

Table 1: Effect of glucose formulation of *T. viride* (seed treatments) on seed germination, root length, shoot length and seedling vigour index (SVI) in Tomato.

Tr. No.	Treatments	Seed germination* (%)	Root Length* (cm)	Shoot Length* (cm)	SVI*
T1	Talc-based *T. viride* at 10g/kg	82.33 (55.43)	5.66	11.80	1598.21
T2	Glucose based *T. viride* at 2.5 g/kg	70.33 (44.70)	4.22	8.43	882.81
T3	Glucose based *T. viride* at 5 g/kg	71.66 (45.78)	4.83	8.74	972.41
T4	Liquid-based *T. viride* at 10 ml/kg	76.33 (49.76)	5.44	9.88	1169.30
T5	Glucose based *T. viride* at 10g/kg	92.66 (66.50)	7.97	14.90	2097.91
T6	Thiram at 2 g/kg	89.33 (63.46)	6.97	13.95	1869.79
T7	Control (Untreated)	56.33 (34.29)	3.59	6.11	546.62
	SE ±	**1.12**	**0.079**	**0.11**	**24.61**
	CD (P = 0.05)	**3.46**	**0.24**	**0.36**	**75.73**

** Mean of three replications, Figures in parenthesis are arcsine transformed values*

The important finding of the present study is, when glucose used as a carrier for the formulation of *Trichoderma viride,* the highest percentage of tomato seed germination, along with maximum root length, shoot length and seedling vigour index were recorded as compared to untreated control, talc as well as a liquid base formulation of the same strain. In the past nobody used glucose as a carrier for the formulation of *Trichoderma viride*. According to Bas *et al* (Dekkers, Schuurmans and Smeekens, 2004) glucose having a stimulatory role in germination but exogenously applied glucose delayed seed germination in arabidopsis plant but interestingly in the present study we reported that if glucose applied along with the *Trichoderma viride* through seed treatment for tomato then it enhances the seed germination. It might happen because fungi require different nutrients at different concentration for growth. Several authors have reported that; the genera *Fusarium, Aspergillus, and Penicillium* use glucose in different metabolic processes (Beyer et al, 2004, Daynes, McGee and Midgley, 2008, Olsson et al, 1994, Panagiotou et al 2008). Soil fungi secrete several enzymes used in cycling and uptake of nutrients. Synthesis of these enzymes depends on environmental conditions as well as the ability of each species and fungus strain (Beatriz de Oliveira Costa and Ely Nahas, 2012).

Addition of carbohydrate (glucose) in the soil with *Trichoderma viride* though seed treatment probably accelerated the fungal growth, increased enzymatic activity and stimulated the tomato seed germination that would not have been happened with talc and liquid base formulations. Talcum powder is the magnesium silicate which has inert in nature, it may not be supported to the growth of *Trichoderma viride* and seed germination never accelerated. In case of liquid base formulation mineral oil used along with water, which might have unfavorable effects on seed germination. Glucose is biodegradable, having neutral pH, most of the fungi utilize it as a source of carbohydrate. According to the Beatriz de Oliveira Costa and Ely Nahas, (2012) the addition of glucose in the soil along with fungus *A. flavus* and *Penicillium* spp. dehydrogenase and amylase activity increased and fungus growth stimulated. They further reported that addition of glucose in the soil increased total carbohydrate content of soil and consumption of total carbohydrate is more pronounced in soil with glucose than in soil without glucose. Glucose is the most widely utilizable carbon source, hence it is used most commonly in number of growth mediums. In the present study, glucose formulation of *T. viride* applied through seed treatment and glucose sugar of that formulations might be a contributory factor in the acceleration of seed germination. The physiological and genetical analysis is necessary to find out the exact role of glucose with *Trichoderma viride* w.r.t. acceleration of seed germination.

CONCLUSION

The present investigation clearly indicated that *Trichoderma viride* enhances germination ability of tomato seeds in all the treatment as compared to untreated but glucose base seed treatments provided highest seed germination, root length, shoot length and seedling vigour index. Glucose base formulation of *T. viride* could be the best approach for plant growth promotion. It is not only cost-effective but eco-friendly and most suitable for organic farming.

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