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Effect of fungicides and bioagents on rhizosphere microbial population, microbial biomass carbon and dehydrogenase activity in soybean (*Glycine max* L. Merrill) soil

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Abstract
The use of fungicides in agriculture, to protect plants from soil borne pathogens, is a common practice. However, there is a dearth of information on the side effects of fungicides on key soil ecological and microbial processes. Effects of different fungicides on soil microbial population, microbial biomass carbon and dehydrogenase activity were investigated. Fungicide effects on bacteria, fungi and actinomycetes were determined on the basis of their growth in respective medium. The population of fungi declined significantly while bacteria and actinomycetes population showed non-significant effect. Most of the fungicides had favorable effect on bacterial population in comparison to the control. Maximum reduction of 21.9 percent in fungal population was found with the use of *Trichoderma viride*. The application of fungicides and bioagents through seed treatment did not have significant effect on the actinomycetes population in rhizosphere soil. Application of most of the fungicides resulted in increase in microbial biomass carbon and dehydrogenase activity. Among the treatments, application of *Pseudomonas fluorescens* registered highest microbial biomass carbon and dehydrogenase activity.

Keywords: Fungicides, bioagents, bacteria, fungi, actinomycetes

Introduction
Fungicides are used in agriculture for a variety of reasons and are applied in various ways; always resulting their entry in the soil in large or smaller amounts, where they may directly or indirectly influence the processes occurring therein. Fungicides tend to inhibit the population of soil borne pathogens when applied through seed treatment. Application of these chemicals sometime leads to decrease in the population of soil flora and fauna. But in general, the fact is that most of the chemicals which are used in crop field can be degraded by soil microorganisms. Microorganisms are of assistance in increasing the soil fertility and plant growth as they are concerned in several biochemical conversions and mineralization actions in soils. The soil microorganisms ensure the permanence of element cycles in nature due to the array of their metabolic behavior. The consequence of their activities is not only mineralization of organic compounds but also the changes of mineral compounds, which have an immense impact on the development of the plants. They form a vital part of the soil food web, therefore, microbial biomass is considered to be a measure of potential microbiological and ecosystem functioning. However, for proper understanding of ecosystem functioning and determining soil disturbances because of various agricultural management practices, microbial activities must also be determined along with microbial biomass carbon. Soil enzymes are recognized as sensitive indicator of soil health and quality. In fact, they have been related to soil physicochemical characters, soil ecology and soil fertility. They catalyze several vital reactions necessary for microbial processes in soil agriculture and described as biological fingerprints of soil. Dehydrogenase is a soil enzyme that oxidizes soil organic matter and is commonly used as a biological indicator of microbial respiratory activity. So, the present investigation was carried to study the influence of applied fungicides and bioagents on *rhizosphere* soil microbial population, microbial biomass carbon and dehydrogenase activity in soybean.

Materials and Methods
A field experiment was conducted during *Kharif* season of 2016 at Pantnagar to study

the effect of selected fungicides and bioagents on the microbial population in rhizosphere of soybean variety PS 1347. The soil of the experimental site was silty clay loam of pH 7.4, having 0.87% organic carbon, 192 kg/ha available nitrogen, 24.6 kg/ha available phosphorus and 160.16 kg/ha available potassium. The experiment was conducted in randomized block design with three replications in 5 x 3.6 m^2 plots. Soybean seed (var. PS 1347) was sown @ 80 kg/ha with a spacing of 45 cm between rows, at 5 cm depth.

The crop was uniformly fertilized with a basal dose of nitrogen (urea), phosphorus (SSP) and potassium (MOP) at 20, 60, 40 kg/ ha, respectively at the sowing time. Plant population was maintained to 40 plants per square meter area. Soybean seed was treated with different fungicides and bioagents. Seed inoculation with *Bradyrhizobium japonicum* culture was done in all the treatments uniformly. There were fourteen treatments i.e. Control (T_1), Carbendazim @ 1.5 g/kg seed (T_2), Mancozeb @ 2.5 g/kg seed (T_3), Thiram @ 2.5g/kg seed (T_4), Captan @ 2.0 g/kg seed (T_5), *Pseudomonas fluorescens* @ 5g/kg seed (T_6), *Trichoderma viride* @ 5g/kg seed (T_7), Carbendazim+Mancozeb @ 3g/kg seed (T_8), Carbendazim+Thiram @ 3g/kg seed (T_9), Carbendazim+Captan @ 3g/kg seed (T_{10}), Mancozeb+Thiram @ 4g/kg seed (T_{11}), Mancozeb+Captan @ 4g/kg seed (T_{12}), Thiram+Captan @ 4g/kg seed (T_{13}), *Pseudomonas fluorescens* + *Trichoderma viride* @ 5g/kg seed (T_{14}).

Pour plate serial dilution method was used for estimating the population of total bacteria, fungi and actinomycetes in soil. The soil was serially diluted and aliquots of suitable dilutions were plated with the appropriate culture medium in triplicate. The culture media used were Thronton's agar media for bacteria, Martin's Rose Bengal streptomycin agar medium for fungi and Munair & Kenknight's medium for actinomycetes. The population of these microorganisms in rhizosphere soil was computed by multiplying the mean colonies with the dilution factor used for computing population.

Viable counts (cfu/g of soil) = Average number of colonies × Dilution Factor ×

$$\frac{1 - \text{moisture}\ \%}{100}$$

Results and Discussion

Population of soil microbes

Application of fungicides and bioagents did not show significant effect on the rhizosphere population of bacteria (Table 1). Bacterial count ranged from 7.61 to 8.50 x 10^6 cfu/g of soil at 50 percent flowering stage (Fig. 1). However, most of the fungicides showed favourable effect on bacterial population in comparison to the control. The application of Carbendazim (T_2), Mancozeb (T_3), Captan (T_5), *Trichoderma viride* (T_7), and combined application of Carbendazim + Mancozeb (T_8), Carbendazim + Thiram (T_9), Mancozeb + Captan (T_{12}) and Thiram + Captan (T_{13}) increased bacterial population in the rhizosphere soil from 1.9 to 6.9 percent. The use of Mancozeb (T_3) recorded maximum number of rhizosphere bacteria (8.5×10^6 cfu/g soil).

The application of *Trichoderma viride* (T_7), combined use of Carbendazim + Captan (T_{10}), Mancozeb + Thiram (T_{11}), Thiram+Captan (T_{13}) and *Pseudomonas fluorescens+Trichiderma viride* (T_{14}) significantly reduced fungal population in rhizosphere soil (Fig. 2). However, the use of fungicides in other treatments also reduced fungal population in rhizosphere soil, but the reductions were non-significant. Maximum reduction of 21.9 percent in fungal population was found with the use of *Trichoderma viride* (T_7). All the treatments, except *Pseudomonas* showed numerical decrease in the fungal population in comparison to control treatment.

The application of fungicides and bioagents through seed treatment did not have significant effect on the actinomycetes population in rhizosphere soil (Fig. 3). Seed inoculation with *Pseudomonas* @ 5 g/kg showed the maximum number of actinomycetes (11.56 x 10^5 cfu/g of soil) rhizosphere soil. However, all the treatments showed numerical increase in actinomycetes population in comparison to control except the combined application of *Pseudomonas fluorescens+Trichoderma viride* @ 5g/kg seed (T_{14}).

The favorable effect on the bacterial population in the rhizosphere due to application of fungicides may be because some fungicides serve as source of carbon and other nutrients when metabolized by soil bacteria. Higher population of bacteria may be attributed due to their tolerance to the action of fungicides. Some systemic fungicides do not exert adverse effect on bacterial population. This is in conformity with the findings of Liu and Hsiang (1994) [8]. These findings also show similarities with the study of Wainwright and Pugh (1974) [13] who observed that application of fungicides at twice the normal rate increased the bacterial population. The results of the present investigation are in agreement with the findings of Gupta *et al.* (1988) [5], Curley and Burton (1975) [3]. Fungicides treated soil harbored less population of fungi in comparison to control. All fungicide treatments showed lower fungal population than control treatment. This is in conformity with other reports (Shukla *et al*. 1987; Colinas *et al*. 1994) [12, 2]. Fungicides i.e. Mancozeb degrades and forms byproducts such as thiurium disulphide, carbon disulphide and ethylene diisocyanele (Gruzdyev *et al*. 1988) [4]. Mancozeb and its degradation product persisted for a longer period. These findings corroborate with that of Ingham (1985) [6] who also observed the reduction of fungal population due to application of fungicides. The highest population of fungi in soil was found in the plot treated with *Pseudomonas fluorescens* @ 5g/kg seed (T_6). This might be due to the phosphate solubilizing ability of *Pseudomonas*, which resulted in enhanced phosphate supply and provide conducive environment in soil for fungi.

Microbial biomass carbon (MBC)

Application of fungicides either alone or in combination significantly increased MBC in soil except combined use of *Pseudomonas fluorescens* + *Trichoderma viride* (T_{14}). The MBC in soil ranged from 217.34 µg/g soil to 365.46 µg/g soil (Table 2, Fig 4). All the treatments registered significantly higher MBC in soil than the treatment having combined application of *Pseudomonas fluorescens* + *Trichoderma viride* (T_{14}). The application of *Pseudomonas fluorescens* (T_6) gave highest MBC of 365.46 µg/g of soil.

Increase in MBC due to application of fungicides may be because of change in the plant physiology which altered root exudation pattern that may have favorable effect on the microbial population. Some fungicides may also serve as the source of nutrients for soil microflora which may enhance the number as well as activity of soil microbes. According to Sethia and Gupta (2013) [11] microbial biomass carbon increased with application of biopesticides like *Pseudomonas fluorescens*.

Dehydrogenase Activity (DHA)

All the treatments with fungicides significantly enhanced dehydrogenase activity in rhizosphere soil at 50 percent flowering stage (Table 2, Fig 5). The highest dehydrogenase activity (200.15 µg TPF/24 h/g oven dry soil) was found with the use of *Pseudomonas fluorescens* (T_6) and the lowest dehydrogenase activity (132.6 µg TPF/24 h/g oven dry soil) was given by *Pseudomonas fluorescens* + *Trichoderma viride* (T_{14}). All the treatments with fungicides were significantly better than combined application of *Pseudomonas fluorescens* + *Trichoderma viride* (T_{14}) by increasing dehydrogenase activity in soil.

Dehydrogenase is considered as an indicator of overall microbial activity because it occurs intracellular in all living microbial cells and is linked with microbial respiratory processes (Burns, 1978) [1]. Dehydrogenase enzyme is known to oxidize soil organic matter by transferring protons and electrons from substrates to acceptors. Dehydrogenase activity is thought to reflect the range of oxidative activities of soil microflora. The increase in DHA in rhizosphere soil indirectly related with the number of soil microorganisms. Application of fungicides may have favorable effect on micro organisms which increases dehydrogenase activity. These findings are in agreement with Madhuri and Rangaswamy (2015) [9] they found that Dhanustin, Dithane M-45 and Contaf had a positive impact on dehydrogenase. The activity of dehydrogenase enzyme in the soil system is very important as it may give indications of the potential of the soil to support biochemical processes which are essential for maintaining soil fertility (Joachim *et al.* 2008) [7]. The soil enzyme activity was found to be influenced by native soil eco-system and fertilizer application, soil dehydrogenase activity, as an indicator of total soil microbial activity, reflects the fertility of soils (Rautaray, 2005) [10].

Table 1: Effect of fungicides and bioagents on rhizosphere microbial population of soybean var. PS1347 at 50 percent flowering stage

Treatments		Bacteria ($\times 10^6$ cfu g^{-1} soil)	Fungi ($\times 10^4$ cfu g^{-1} soil)	Actinomycetes ($\times 10^5$ cfu g^{-1} soil)
T_1	Control	7.95	10.85	10.04
T_2	Carbendazim @ 1.5 g/kg seed	8.10	10.35	10.06
T_3	Mancozeb @ 2.5 g/kg seed	8.50	10.42	10.58
T_4	Thiram @ 2.5 g/kg seed	7.89	10.55	10.78
T_5	Captan @ 2.0 g/kg seed	8.09	10.28	10.67
T_6	*Pseudomonas fluorescens* @ 5 g/kg seed	7.90	12.31	11.56
T_7	*Trichoderma viride* @ 5g/kg seed	8.16	8.90	10.54
T_8	Carbendazim+Mancozeb @ 3g/kg seed	8.02	9.95	10.18
T_9	Carbendazim+Thiram @ 3g/kg seed	8.17	10.64	10.08
T_{10}	Carbendazim+Captan @ 3g/kg seed	7.86	9.86	10.15
T_{11}	Mancozeb+Thiram @ 4g/kg seed	7.89	9.84	10.11
T_{12}	Mancozeb+Captan @ 4g/kg seed	8.16	9.89	10.12
T_{13}	Thiram+Captan @ 4 g/kg seed	8.14	9.85	10.16
T_{14}	*Pseudomonas fluorescens+Trichoderma viride @ 5g/kg seed*	7.61	9.76	10.02
CD (p=0.05)		NS	0.93	NS

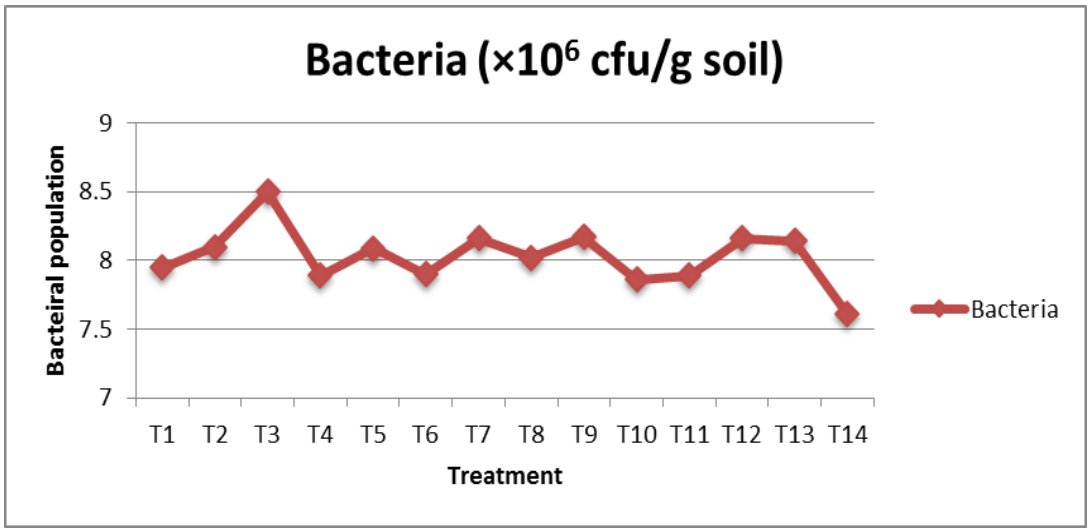

Fig 1: Effect of fungicides and bioagents on bacterial population in soil

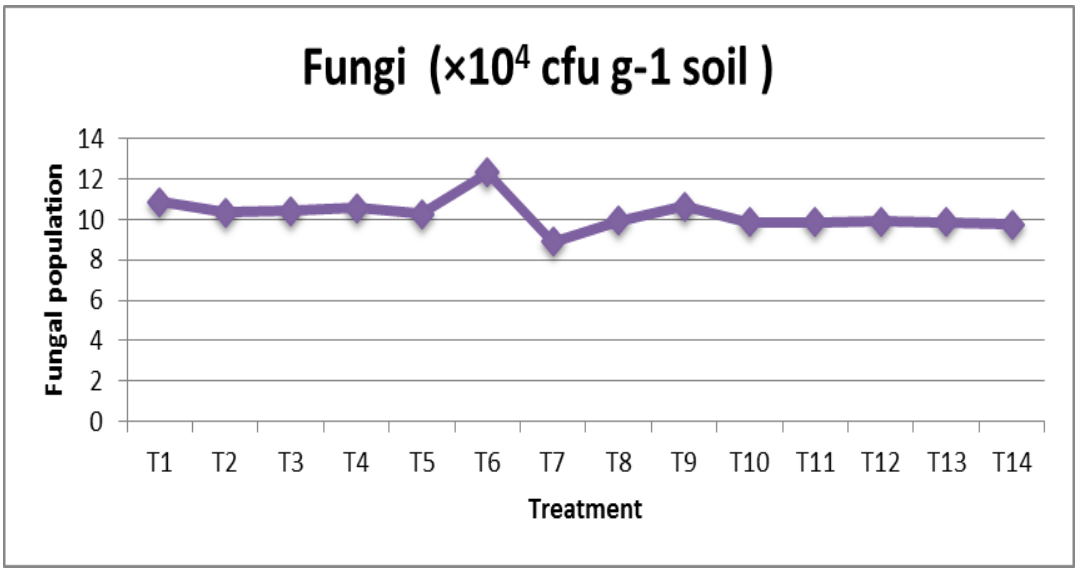

Fig 2: Effect of fungicides and bioagents on fungal population in soil

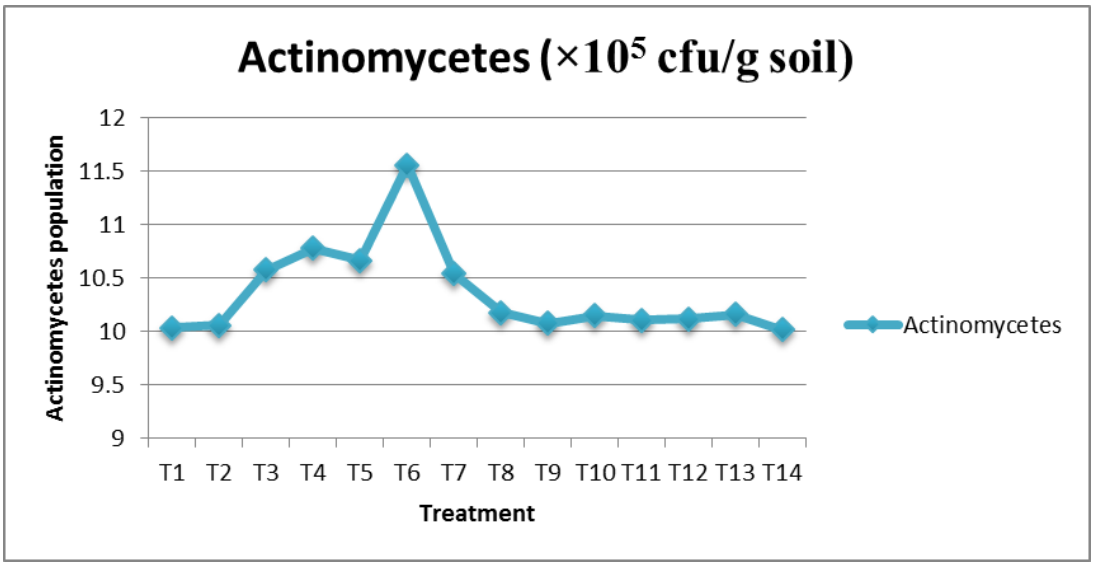

Fig 4.12: Effect of fungicides and bioagents on Actinomycetes population in soil

Table 2: Effect of fungicides and bioagents on microbial biomass carbon and dehydrogenase activity in soil at 50 percent flowering stage

Treatments		MBC (µg/g soil)	DHA (µg TPF/24 h/g oven dry soil)
T_1	Control	217.34	131.15
T_2	Carbendazim @ 1.5 g/kg seed	344.95	173.70
T_3	Mancozeb @ 2.5 g/kg seed	353.79	170.37
T_4	Thiram @ 2.5 g/kg seed	265.38	169.26
T_5	Captan @ 2.0 g/kg seed	306.05	168.33
T_6	*Pseudomonas fluorescens* @ 5 g/kg seed	365.46	200.15
T_7	*Trichoderma viride* @ 5g/kg seed	358.05	198.48
T_8	Carbendazim+Mancozeb @ 3g/kg seed	284.50	166.85
T_9	Carbendazim+Thiram @ 3g/kg seed	352.53	163.89
T_{10}	Carbendazim+Captan @ 3g/kg seed	276.30	157.60
T_{11}	Mancozeb+Thiram @ 4g/kg seed	256.99	142.80
T_{12}	Mancozeb+Captan @ 4g/kg seed	325.05	162.41
T_{13}	Thiram+Captan @ 4 g/kg seed	328.27	158.53
T_{14}	*Pseudomonas fluorescens+Trichoderma viride @ 5g/kg seed*	234.65	132.26
CD (p=0.05)		18.79	10.34

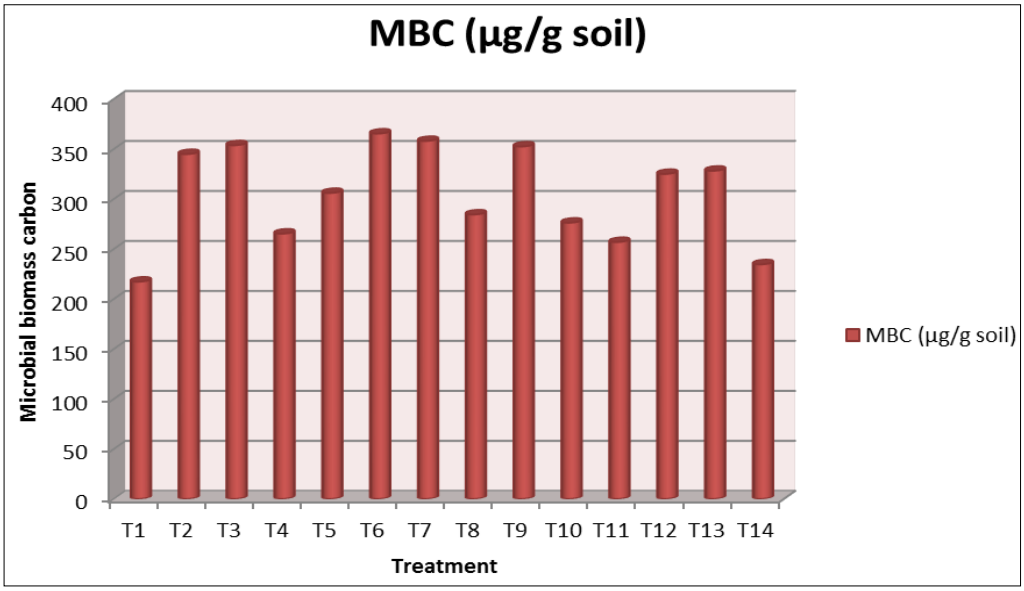

Fig 4: Effect of fungicides and bioagents on microbial biomass carbon in soil

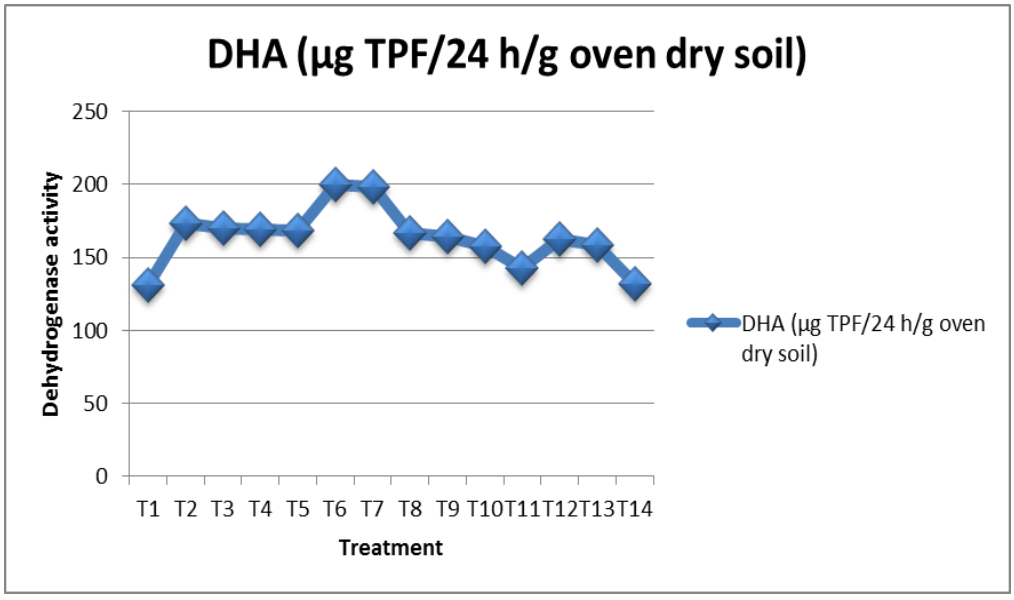

Fig 5: Effect of fungicides and bioagents on Dehydrogenase activity in soil.

Conclusion

The study was able to reveal that the fungal population in rhizosphere soil significantly decreased with the use of fungicides but the population of bacteria and actinomycetes increased however, the increase was not significant. Microbial biomass carbon and dehydrogenase activity of soil were significantly affected by seed treatment through fungicides.

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