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INVESTIGATION OF BIOLOGICAL CONTROL OF TRICHODERMA FORMULATIONS AND ITS MUTANT TYPE RELATED TO CHEMICAL TREATMENTS IN THE CONTROL OF SOYBEAN CHARCOAL ROTS DISEASE

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ABSTRACT

In order to study biological control of soybean charcoal rot disease using multiple mutant *Trichoderma* formulations with gamma ray, an experiment under greenhouse conditions was performed in the years 2017 and 2018 at Karaj Nuclear Agriculture Research Institute. Beside control the experiment consisted of 16 treatments including chemical and commercial fungicides and inoculation of mutant and non-mutant soybean with *Trichoderma* fungi. The results showed that the effect of treatments was significant for all morphological and physiological characteristics of soybean. The use of *Trichoderma* treatments had a positive influence on plant morphological characteristics such as pod number, stem dry weight, stem length, number of branches and physiological traits such as peroxide, chlorophyll a, chlorophyll b and carotenoids. As a result, they increased the yield so that the highest average of 11.75 g per pot was registered to the seed coating treatment with wild *Trichoderma* (PW). The use of fungal treatments increases proline and malondialdehyde content and the incidence of disease is reduced by using *Trichoderma*.

Keywords: biological control, gamma ray, mutant *Trichoderma*, soybean charcoal rot disease.

INTRODUCTION

Soybeans (*Glycine max* L.) are susceptible to a large number of pathogens and are most damaged by *Macrophomina phaseolina* (Tassi) Goid, which targets seedlings and roots (Jana et al., 2003). Charcoal rot is the most important fungal disease of soil borne in soybean. Fungi *Macrophomina phaseolina* (Tassi) Goid are the cause of charcoal rot among soil borne pathogens which is one of the most important pathogens of various plants in warm regions. Biological control of useful organisms or their products included resulting in reducing the negative effects of the plant pathogens (Vinale et al., 2008). Because fungus is soil borne and has high power of saprophytic, it is not possible to easily control and fungicides cannot control it properly (Mengistu et al., 2011). So researchers have been using biological control and have brought positive results (Gajera et al., 2012; Gupta et al., 2012). Density and survival of *T. harzianum* on several substrates showed that the use of these formulations significantly reduces wilting in beans and crown rot in peanuts (Singh et al., 2007). Haggag (2002) stated that mutants compared to wild type have higher levels of proline and hydroxyproline, sodium and phenolic compounds and also found that the mutants produced active metabolites such as chitinases, cellulases, β-galactosidase, as well as a number of antibiotics such as trichodermin, Gliotoxin, and gliovirins, which mainly reduced the incidence of the disease. This study was performed to evaluate the efficacy of several mutagenic *Trichoderma* formulations with gamma ray in the biological control of *Macrophomina phaseolina* (Tassi) Goid in soybean.

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MATERIAL AND METHODS

In order to evaluate the efficacy of several mutagenic *Trichoderma* formulations with gamma ray in the biological control of *Macrophomina phaseolina* (Tassi) Goid in soybean, greenhouse experiments in the form of randomized completely design carried out in four replications for morphological traits and three replications in 2017 and 2018 in Karaj Nuclear Agriculture Research Institute.

Experimental treatments were included:

1- Control (C);
2- Soybean + Powder formulation miscible with wild isolate soil (PW);
3- Soybean + Powder formulation miscible with mutant isolate soil (PM);
4- Soybean + Granule formulation of wild isolates (GW);
5- Soybean + Granule Formulation of Mutant Isolate (GM);
6- Soybean coated with wild isolate (CW);
7- Soybeans coated with mutant isolate (CM);
8- Soybean + *Macrophomina phaseolina* (CMP);
9- Soybean + *Macrophomina phaseolina* + wild isolate powder formulation (GWP);
10- Soybean + *Macrophomina Phasolina* + Mutant Isolation Powder Formulation (PWP);
11- Soybean + *Macrophomina phasolina* + wild Isolate Granule Formulation (CMP);
12- Soybean + *Macrophomina phasolina* + Mutant Isolate Granule Formulation (GMP);
13- Soybean coated with wild isolate + *Macrophomina phasolina* (PMP);
14- Soybean coated with mutant isolate + *Macrophomina phasolina* (pathogen);
15- Soybean + *Macrophomina phasolina* + *Trichoderma* Commercial formulation (Commercial);
16- Soybean + *Macrophomina phasoline* + Chemical fungicide (Chemical);

In order to obtain the most harmful isolates, *M. phaseolina* isolated from the pathogenicity, soil tests were carried out from the fields of soybean infected areas and the fungus pathogenicity was identified and purified. Farms with endemic contamination were sampled from five provinces of Khuzestan, Qazvin, Alborz, Khorasan Razavi and Tehran. *Trichoderma* isolates were isolated on the selected medium, purified and identified. Five species of fungi, *T. virens, T. viride, T. atroviride* and *T. koningii, Trichoderma harzianum*, using several major soil fungal pathogens (*Macrophomina phaseolina, Rhizoctonia solani, Pythiumultimum, Fusarium solani, Fusarium oxysinporum*) on a lab scale with cross-culture tests and volatiles and secondary metabolites were studied and the dominant species in terms of biocontrol power was identified for each pathogenicity. Dosing operation was performed on the spore suspension of superior species and 250 g was determined. Radiation operation performed at the optimal dose for all five species (500 beam spores from each species) and based on multiple replanting stability of morphological traits, superior isolates were selected in terms of growth rate and colony germination and ability to reproduce (sporulation rate) and spore germination rate. Among these mutants selected using the cross-culture test with the pathogenicity, the top 20 candidate mutants were selected for the production of biological toxins. These mutants were examined using two molecular markers STS and RAPD and their protein profile and enzyme activity (affecting antagonism activity) of chitinolytic and cellulite were consider and isolates with antagonistic power and enzymatic production higher than their wild type. Afterward, the biocontrol power of all selected mutants in the laboratory phase was investigated under greenhouse conditions on specific hosts of each plant pathogenicity for two consecutive years. After that, various agricultural wastes were used as substrates for solid bed cultivation of these selected mutants to determine the optimal environment and suitable conditions for growth and production of mutant spores. The best formulation was selected after observed the amount of controlling of formulations in the greenhouse. Measured traits included morphological characteristics (disease incidence, seed yield, stem dry weight, plant height, number of pods per plant and number of branches) and physiological characteristics (peroxide, chlorophyll a, chlorophyll b, carotenoids, malondialdehyde). Statistical analysis of data was performed using SAS software and comparison of mean data was performed through Duncan's multiple range tests at 5% probability level and then the figures were drawn using Excel software.

RESULTS AND DISCUSSION

Grain yield:

The results showed that the use of *Trichoderma* treatments increased grain yield in most of the treatments that used *Trichoderma*. The highest grain yield were obtained in seed coating treatment with wild *Trichoderma* (PW) (11.75 g) compare to commercial *Trichoderma* treatments and contaminated soil (Figure 1). Charcoal rot reduces plant weight, root volume and root weight. Damage to the root system is more evident during pod formation and seed filling when there is more competition for water and food. Because the diseased plant has a shorter root system and eventually the seeds produced are thin and light therefore formed in smaller numbers. Elad (1996) reported that Inoculum isolated from *T. harzianum* on wheat straw added to the soil significantly prevents the occurrence of root diseases in beans (with *R. solani, S. rolfsi* or both). Also *Trichoderma* inoculum on wheat straw in healthy soils (not contaminated with the disease agent) increases plant growth. Fungi produce more grain yield by increasing plant growth by providing essential growth elements such as phosphorus, increasing photosynthetic pigments, number of leaves, plant height, number of pods that are yield component. Also, some fungal treatments increase the content of proline and peroxide and decrease the malondialdehyde content due to the plant's resistance to the pathogen and prevent reduction of the yield.

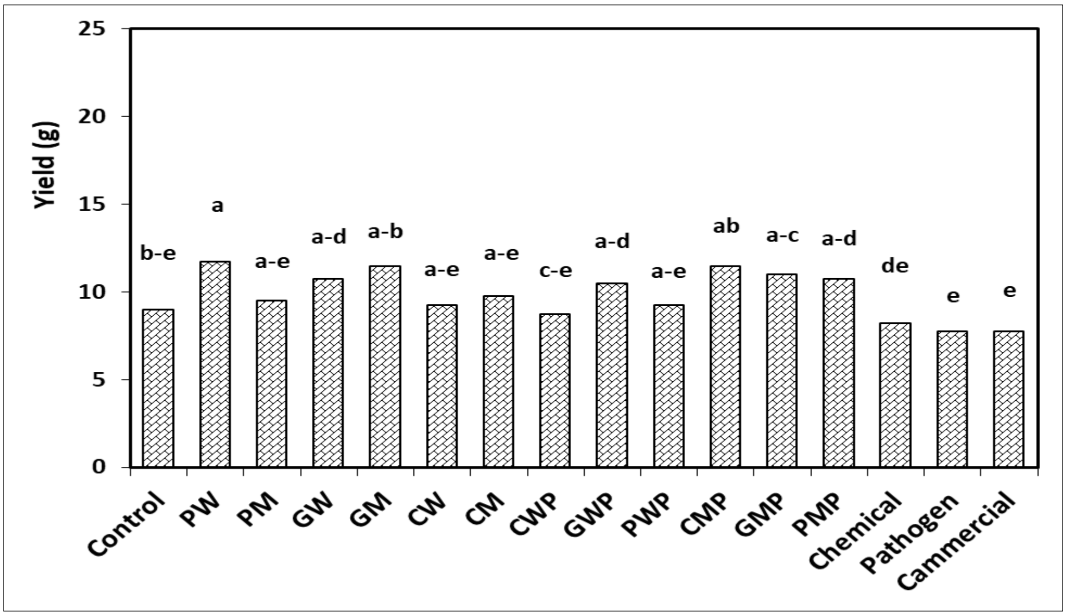

Figure 1. The yield in different fungal treatments

Number of pods:

The results of mean comparing of pods number showed that the use of *Trichoderma* treatments in most treatments increased the number of pods in soybean so that the highest average number of pods belonged to PWP (43) and the lowest average number of pods showed pathogen treatments (18.75) and commercial *Trichoderma* (Table 1). *Mycorrhiza* and *Trichoderma* can also improve plant growth and reduce application of fertilizers and fungicides (Nzanza et al., 2011). Fungi by providing optimal growing conditions increased stem and branch growth, therefore provide more storage material to produce more pods.

Stem dry weight:

The results of mean comparison showed that the highest mean dry weight of stem in GM (30 g) was related to most of the treatments that used *Trichoderma*. In commercial *Trichoderma* and chemical treatments and contaminate soil (15.25 g), the dry weight of the stem decreased even compared to the control treatment (Table 1). The use of fungi with solution and making nutrients available to the plant has increased the growth and length of the stem and increased the number of branches in the soybean plant, resulting in increased dry weight of the stem. The researchers showed that use of conventional fertilizers, plant growth was not affected by *Mycorrhiza* but the addition of *Trichoderma* showed a significant response (Martinez-Medina et al., 2011).

Stem length:

The results show that CW treatment (57.75 cm) has the highest stem length and there is a slight difference between other treatments (Table 1). *Trichoderma* is strongly influenced by plant physiology to changes in gene expression, changes in gene expression in the shoot is greater than the root (Shoresh et al., 2010). These changes in gene expression by plant in general improve plant performance. When phosphorus is limited factor, *Trichoderma* can increase the solubility of phosphorus subsequently stimulate the production of growth hormones and plant growth (Richardson et al., 2011). The use of *Trichoderma* increased photosynthetic pigments and growth hormones and increased the vegetative growth of soybean and after that the stem length increased.

Number of branches:

The results showed that the use of *Trichoderma* fungal treatments increased the number of branches in most fungal treatments. The highest mean was related to GWP treatment (6.25). This treatment was able to produce the largest number of branches by controlling charcoal rot (Table 1). Pathogen treatments, commercial *Trichoderma* treatment and chemical toxin treatment, PM and control had the lowest number of branches compared to other treatments (Table 1).

Table 1. Mean comparison of morphological traits in soybean

	Number of pods	Steam dry weight (g)	Steam length (cm)	Number of branches
C	35.75 a-c	24.75 a-c	38.25 cd	4.00 d
PW	34.50 a-c	24.00 a-c	45.50 b	5.50ab
PM	32.50 a-c	23.50 b-d	37.75 d	4.00 d
GW	39.75 a-c	29.00ab	34.50 d	5.00bc
GM	38.75 a-c	30.00 a	46.25 b	5.50ab
CW	35.25 a-c	25.25 a-c	57.75 a	4.75 b-d
CM	41.00ab	26.25 a-c	43.50bc	5.50ab
CWP	40.00 a-c	28.00 a-c	34.50 d	5.50ab
GWP	35.75 a-c	22.00 c-e	33.25 d	6.25 a
PWP	43.00 a	26.75 a-c	34.50 d	5.50ab
CMP	31.75 a-c	26.50 a-c	44.00bc	5.25 b
GMP	38.75 a-c	27.75 a-c	34.75 d	4.75 b-d
PMP	39.00 a-c	26.00 a-c	36.75 d	5.50ab
Chemical	26.75 c-d	18.25 d-f	43.75bc	4.00 d
Pathogen	18.75 d	15.25 f	36.75 d	4.00 d
Commercial	28.00 b-d	17.50ef	36.25 d	4.25 cd

Fungi also increased shoot growth by increasing the solubility of essential elements. Researchers reported that use of *Trichoderma* species in the culture medium cause to the solubility of nutrients in the *Rhizosphere* environment, the secretion of growth hormones and take advantage of the side effects (Pill et al., 2009). The use of *Trichoderma* increased the amount of important pigments of photosynthesis such as chlorophyll a and b, carotenoids and growth hormones, and increased vegetative growth, stem length and number of branches that are functional components.

Disease Incidence:

The results showed that the highest incidence of the disease was related to pathogen treatment (4.5%) and the lowest percentage was related to control treatments (1%). Most treatments were chemical to use *Trichoderma* mutant, commercial *Trichoderma* as well as fungicide treatment (Figure 2). The use of different treatments of *Trichoderma* formulation reduces the percentage of disease. Several studies indicate that interaction between two groups of microorganisms may be effective for better for plant growth and development and control of some diseases (Martinez-Medina et al., 2011). Use of all disease control methods such as *Trichoderma* mutant, commercial *Trichoderma* and chemical fungicide treatments by reducing ROS and destructive biomarkers such as malondialdehyde by increasing proline and peroxide and increasing pigment production to increase photosynthesis make soybean resistant to charcoal rot and incidence of disease in these treatments was reduced compared to the pathogen treatment.

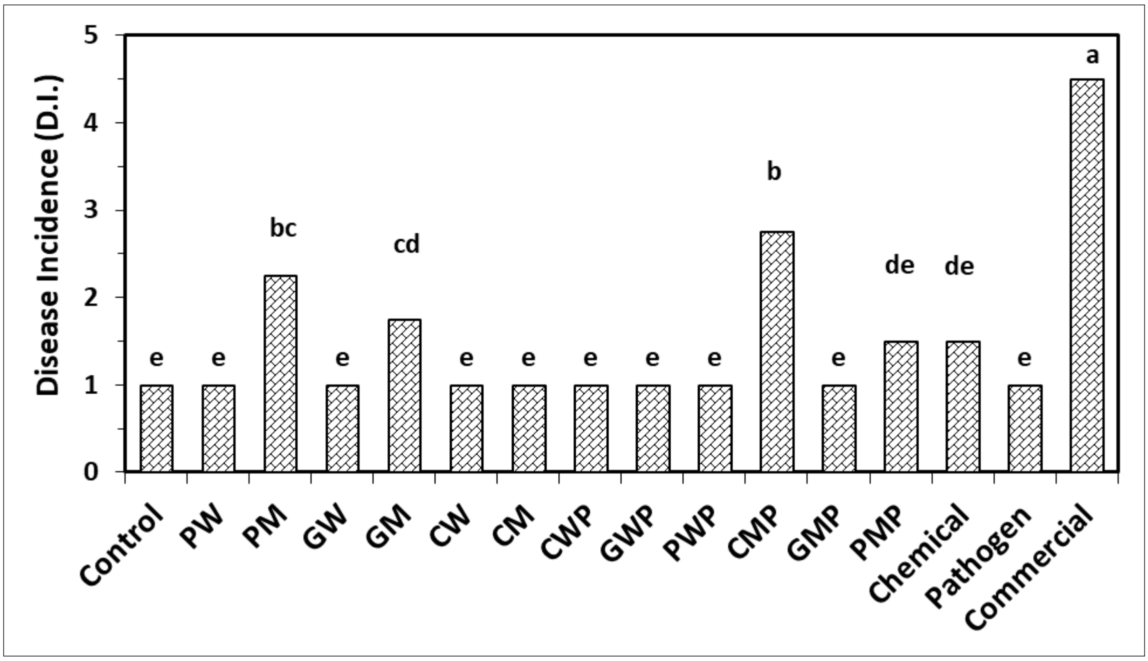

Figure 2. Comparison of the average incidence of disease in different fungal treatments

Peroxide:

The results showed that the highest amount of peroxide was related to most fungal treatments so that the highest average peroxide was related to CWP treatment (106.93 U/g fw leaf). The results also showed that the lowest mean was related to the fungal treatment of GM (29.93 U/g fw leaf) which were at the same statistical level with PWP and commercial *Trichoderma* treatments (Table 2). According to the comparison of means, it can be said that the treatments using GWP, CMP, and CWP had the best role in improving and increasing the level of peroxide activity, respectively, and caused the control of charcoal rot disease. Fungi stimulate the production of peroxide as a defense factor that reduces the destructive effects of the disease. Catalase and peroxidase are the two most important antioxidants that break down hydrogen peroxide (H_2O_2) into water and oxygen molecules (Yang and Poovalah, 2002). In chloroplasts, it detoxifies the H_2O_2 produced by ascorbate peroxidase through the glutathione ascorbate cycle. In fact, APX, with its high adhesion to H_2O_2, can help eliminate plant poisoning (Tewari et al., 2013).

Chlorophyll a:

The results of the mean chlorophyll a showed that all fungal and non-fungal treatments had more chlorophyll a to control charcoal rot than pathogen treatment (0.79 mg/g fw leaf), the highest mean of chlorophyll a was related to PW treatment (4.29 mg/g fw leaf) (Table 2). All disease control methods and even control produce more photosynthesis by producing more chlorophyll a, resulting in higher growth, higher yield, and a stronger immune system and more resistance compare to pathogen treatment. The researchers also observed increased concentrations of chlorophyll in cucumber by application of *Trichoderma* treatment (Lo and Lin, 2002). Fungi by increasing pigments, increase growth, yield components (stem length, number of branches, number of pods) and thus increase yield in soybean and also cause resistance to charcoal rot.

Chlorophyll b:

The results of mean comparison showed that the highest mean of chlorophyll b was related to some fungal treatments so that the highest mean was related to fungal treatment GM (1.16 mg/g fw leaf) and the lowest amount of chlorophyll b was related to fungal treatment GWP (0/34 mg/g fw leaf) which were at the same statistical level with GW, CM, CWP, CPM, GWP and pathogen treatments (Table 2). High chlorophyll content in plants inoculated with fungi can be due to a positive relationship between phosphorus concentration and chlorophyll content in inoculated plants (Zarea et al., 2012).

Fungi are also involved in affecting important proteins in the process of photosynthesis and the Calvin cycle and increasing their expression and play an effective role in maintaining and sustaining photosynthesis (Sepehri, 2009). Decreasing the amount of chlorophyll in fungal diseases has a direct effect on photosynthesis. The use of *Trichoderma* fungi increased the amount of chlorophyll and increased photosynthesis, resulting in greater control of the disease.

Carotenoids:

According to the results of this study, the highest amount of carotenoids was obtained from PW fungal treatment (1.18 mg/g fw leaf). The amount of carotenoid pigment in all mutant, chemical, commercial and even control methods was higher than the pathogen treatment (0.20 mg/g fw leaf) (Table 2).

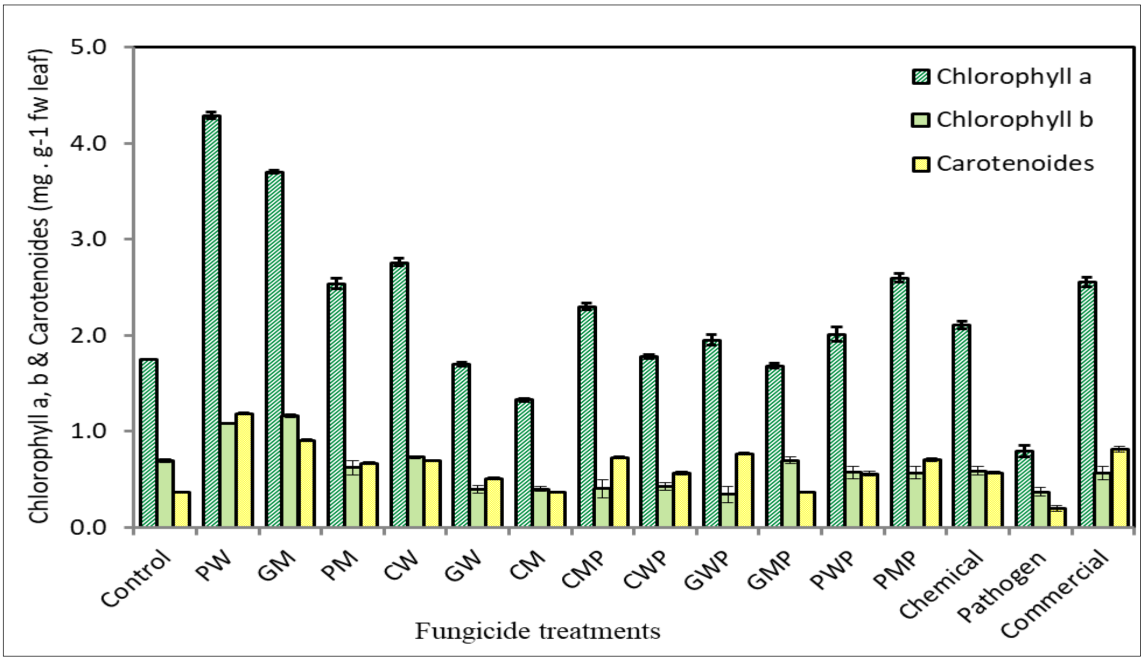

Figure 3. Mean comparison of chlorophyll a and b and carotenoids in different fungal treatments

Carotenoids as biological antioxidants play a very important role in the protection of plant tissue and their absence may cause severe photooxidative damage to plant tissues (Gill and Tuteja, 2010). The use of fungal treatments increases carotenoid pigment by making essential growth elements such as phosphorus available. In general, by examining the three pigments chlorophyll a, chlorophyll b and carotenoids, it can be concluded that the highest mean of these three pigments was related to PW and GM treatments (Figure 3). According to the results, the treatment using charcoal rot pathogen reduced chlorophyll a, chlorophyll b and carotenoids. The disease disorders the growth process of the plant and reduced the amount of pigments. All control methods, especially PW and GM fungal treatments, help plant resistance against pathogens by increasing pigments and increasing photosynthesis.

Proline:

The results showed that some fungal treatments increased the amount of proline in soybean, but the highest mean proline content was related to the chemical toxin treatment (1/23 micro g/g fw leaf) which was at the same level with the fungal treatment PM and CM. It also showed that the lowest proline content belonged to GWP fungal treatment (0/48 micro g/g fw leaf) (Table 2). Proline acts as a potent destroyer of ROS (reactive oxygen species) and prevents cell death by free radicals (Chen and Dickman, 2005). Proline accumulation occurs in response to many biological stresses such as fungal contamination (Slama et al., 2006). The results of this study indicated that some fungal treatments with the highest proline levels were able to have higher yield. Therefore proline increased yield by reducing the destructive effects of the pathogen.

Malondialdehyde:

The results of mean comparison of malondialdehyde amount showed that the use of fungal treatments reduced the production of malondialdehyde in soybean so that the lowest mean was related to GWP (1.15 ng/g fw leaf). Pathogen treatment (2.14 ng/g fw leaf) by increasing free radical oxygenation causes peroxidation of the cytoplasmic membrane and increases this biomarker (Table 2).

Table 2. Mean comparison of physiological traits in soybean

	Peroxidase activity (U/g fw leaf)	Chlorophyll a (mg/g fw leaf)	Chlorophyll b (mg/g fw leaf)	Carotenoides (mg/g fw leaf)	Proline (micro g/g fw leaf)	MDA (ng/g fw leaf)
C	37.60 i	1.752 hi	0.69 bc	0.37 i	0.53 g	1.68 b
PW	62.72 e	4.29 a	1.08 a	1.18 a	0.51 g	1.42 de
PM	50.58 g	2.54 d	0.62 cd	0.67 f	1.19 a	1.56 c
GW	54.78 f	1.70 hi	0.39 e	0.51 h	0.96 c	1.38 d-f
GM	29.93 k	3.70 b	1.16 a	0.90 b	1.11 b	1.57 c
CW	50.21 g	2.76 c	0.73 b	0.70 e	0.69 f	1.47 d
CM	76.53 d	1.33 j	0.40 e	0.36 i	1.23 a	1.35 ef
CWP	106.93 a	2.24 e	0.42 e	0.71 e	1.10 b	1.27 g
GWP	85.19 c	1.95 g	0.34 e	0.77 d	0.48 g	1.15 h
PWP	31.50 k	2.01 g	0.57 d	0.58 g	0.91 cd	1.47 d
CMP	92.91 b	1.78 h	0.42 e	0.56 g	0.56 g	1.42 g
GMP	41.69 ij	1.68 i	0.70 bc	0.36 i	0.65 f	1.26 g
PMP	45.85 h	2.60 d	0.57 d	0.70 e	0.86 de	1.25 de
Chemical	39.32 ij	2.10 f	0.59 d	0.57 g	1.23 a	1.38 d-f
Pathogen	47.22 h	0.79 k	0.36 e	0.20 j	0.78 e	2.14 a
Commercial	32.82 k	2.58 d	0.55 d	0.82 e	0.67 f	1.31 fg

Malondialdehyde (MDA) is a small product but stable lipid peroxidation that has been created from analysis of unstable peroxides of unsaturated fatty acids (Tug et al., 2004). Malondialdehyde (MDA) is a final product of unsaturated lipid peroxidation of cell. Therefore, it is used as a suitable biomarker to determine the amount of lipid peroxidation due to oxidative stress in the cell (Sofo et al., 2004). Fungal treatments by increasing photosynthetic pigments and producing compounds such as peroxide and proline reduced the production of compounds such as malondialdehyde which helped control charcoal rot and ultimately prevented soybean production decline.

CONCLUSIONS

The use of new formulations of *Trichordema* treatments with increasing antioxidants and photosynthetic pigments and reducing ROS and destructive biomarkers such as malondialdehyde increases plant height, number of branches, number of pods in soybean and makes the plant resistant to charcoal rot and consequently cause to increased grain yield.

REFERENCES

Chen, C., and Dickman, M.B., 2005. *Proline suppresses apoptosis in the fungal pathogen Colletotrichumtrifolii*. Proc. Natl. Acad. Sci. USA, 102(9): 3459-3464.

Elad, Y., 1996. *Mechanisms involved in the biological control of Botrytis cinerea incited diseases*. European Journal of plant pathology, 102: 719-732.

Gajera, H.P., Bambharolia, R.P., Patel, S.V., Khatrani, T.J., Goalkiya, B.A., 2012. *Antagonism of Trichoderma spp. against Macrophominaphaseolina: Evaluation of coiling and cell wall degrading enzymatic activities*. Journal of Plant Pathology and Microbiology, 3: 149.

Gill, S.S., and Tuteja, N., 2010 *Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants*. Plant Physiology and Biochemistry, 48: 909-930.

Gupta, G.K., Sharma, S.K., Ramteke, R., 2012. *Biology, epidemiology and management of the pathogenic fungus Macrophominaphaseolina (Tassi) Goid with special reference to charcoal rot of soybean [Glycine max (L.) Merrill]*. Journal of Phytopathology, 160(4): 167-180.

Haggag, W.M., 2002. *Induction of hyperproducing chitinase Trichoderma mutants for efficient biocontrol of Botrytis cinerea on tomato and cucumber plants growing in plastic houses*. Arab J. Biotech., 5(2): 151-164.

Jana, T., Sharma, T., Prasad, R.D., Arora, D.K., 2003. *Molecular characterization of Macrophominaphaseolina and Fusarium* *species by a single primer RAPD technique*. Microbiological Research, 158: 249-257.

Lo, C.T., and Lin, C.Y., 2002. *Screening strain of Tricoderma spp for plant growth enhancement in Taiwan*. Plant Pathology bulletin, 11: 215-220.

Martinez-Medina, A., Antonio, R., Alfonso, A., Jose, A.P., 2011. *The interaction with arbuscularmycorrhizal fungi or Trichodermaharzianum alters the shoot hormonal profile in melon plants*. Phytochemistry, 72: 223-229.

Mengistu, A., Smith, J.R., Ray, J.D., Bellaloui, N., 2011. *Seasonal progress of charcoal rot and its impact on soybean productivity*. Plant Disease, 95: 1159-1166.

Nzanza, B., Diana, M., Puffy, S., 2011. *Tomato (Solanumlycopersicum L.) seedling growth and development as influenced by Trichodermaharzianum and arbuscularmycorrhizal fungi*. African Journal of Microbiology Research, 5: 425-43.

Pill, W.G., Collins, C.M., Glodberger, B., Gregory, N., 2009. *Responses of non-primed or primed seeds of market more 76' cucumber (Cucumis sativas L.) slurry coated with Trichoderma species to planting in growth media in fested with Pythiumaphanidermatum*. Scientia Horticulturae, 121: 54-62.

Richardson, A.E., Lynch, J.P., Ryan, P.R., Delhaize, E., Smith, F.A., Smith, S.E., Harvey, P.R., Ryan, M.H., Veneklaas, E.J., Lambers, H., Oberson, A., Culvenor, R.A., Simpson, R.J., 2011. *Plant and microbial strategies to improve the phosphorus efficiency of agriculture*. Plant and Soil, 349: 121-156.

Sepehri, M., 2009. *Identification of molecular mechanisms of resistance induced by the fungus (Piriformospora indica) to barley under salinity and drought stress*. PhD Thesis in Soil Science Engineering, University of Tehran, Iran.

Shoresh, M., Mastouri, F., Harman, G., 2010. *Induced systemic resistance and plant responses to fungal biocontrol agents*. Annual Review of Phytopathology, 48: 21-43.

Singh, A., Srivastava, S., Singh, H.B., 2007. *Effect of substrates on growth and shelf life of Tricodermaharzianum and its use in biocontrol of diseases*. Bioresource Technology, 98: 470-473.

Slama, I., Messedi, D., Ghnaya, T., Savoure, A., Abdelly, C., Hnaya, T., Savoure, A., Abdelly, C., 2006. *Effects of water deficit on growth and proline metabolism in Sesuviumportulacastrum*. Environ. Exp. Bot., 56(3): 8-231.

Sofo, A., Dichio, B., Xiloyannis, C., Masia, A., 2004. *Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during rewatering in olive tree*. Plant Sci., 166: 293-302.

Tewari, R.K., Hadacek, F., Sassmann, S., Lang, I., 2013. *Iron deprivation-induced reactive oxygen species generation leads to nonautolytic PCD in Brassica napus leaves*. Environmental and Experimental Botany, 91(100): 74-83.

Tug, T., Karatas, F., Terzi, S.M., 2004. *Antioxidant vitamins (A, C and E) and malondialdehyde levels in acute exacerbation and stable periods of patientswith chronic obstructive pulmonary disease*. Clin. Investig. Med., 27: 123-128.

Vinale, F., Sivasitamparam, K., Ghisalberti, E.L., Marra, R., Woo, S.L., Lorito, M., 2008. *Trichodermaplant-pathogen interactions*. Soil Biology and Biochemistry, 40: 1-10.

Yang, T., and Poovalah, B.W., 2002. *Hydrogen Proxide homeostasis: Activation of plant catalase by calcium-calmodulin*. PENAS, 99(6): 4097-4102.

Zarea, M.J., Hajinia, S., Karimi, N., Mohammadi Goltapeh, E., Rejali, F., Varma, A., 2012. *Effect of Piriformospora indica and Azospirillum strains from saline or non-saline soil on mitigation of the effects of NaCl*. Soil Biology and Biochemistry, 45: 139-146.