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Bio-fertilizers and Systemic Acquired Resistance in Fusarium Infected Wheat

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ABSTRACT

Bio-fertilizers have been introduced as an alternative to chemical fertilizers for plant growth and health. The objective of this pot culture experiment was to evaluate the effects of three Bio-fertilizers in single and mixed form containing arbuscular mycorrhizal fungus (*Glomus intraradices*), N+V on growth, yield components, and expression of some defense response genes in wheat infected with *Fusarium oxysporum*. Real time PCR was performed to determine the gene expression levels of *β-1,3-glucanase*, *Oxalate Oxidase,* and *Chitinase* genes. The application of bio-fertilizers significantly increased all studied parameters, except spike length, in infected plants. The highest shoot dry weight was found in Nitroxin+Vermicompost (N+V) treatment and the highest plant height, grain number, 100 grain weight, and biological yield was observed in treatment Mycorrhizal+Vermicompost (M+V). The use of bio-fertilizer resulted in the highest expression level of *β-1,3-glucanase* gene .The *Chitinase* gene showed the lowest expression level in all treatments. Our results indicate that vermicompost application could influence the improvement of mycorrhizal colonization and development of external hyphae.

Keywords: Bio-fertilizer, Fusarium head blight, Gene expression, Sustainable agriculture.

INTRODUCTION

Plants can be affected by environmental stresses (biotic and abiotic) and then respond to them like other living organisms (Allen, 1995). Biotic stresses are one of the most limiting factors which reduce crop yields. Fusarium Head Blight (FHB) or scab, caused by *Fusarium graminearum* is a destructive disease of bread wheat (*Triticum aestivum* L) and barley (*Hordeum vulgare* L.) (Rudd *et al.*, 2001; Tóth *et al.,* 2008). This disease reduced yield, seed quality, kernel weight, destroyed storage proteins and contamination with mycotoxin (Snijders, 1990). Several mycotoxins producing different cancer in human are frequently associated with infected cereal grains by scab fungi *(*Lemmens *et al.,* 2005; Snijders and Perkowski, 1990). Various methods such as fungicides application, integrated pest management system and the use of resistant cultivars have been used to control head blight (Parry *et al*., 1995; Pirgozliev *et al.,* 2003). The use of resistant cultivars with remarkable agronomic parameters is one of the best ways to disease control (Gilbert and Tekauz, 2000). Nutrients are a most important limitation to growth and development of plants (Fry, 2012). The use of fertilizers is an important factor in improving soil-nutrients availability reducing plant disease control (Huber, 1981). The effect of five different types of nitrogen fertilizers (organic and inorganic) on scab disease have shown that nitrogen application at early stage of plant growth significantly reduced fungal development in infected plants (Lemmens *et al.,* 2004) but had no significant effect on Deoxynivalenol (Glick *et al.* 2001) and

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Nivalenol (NIV) toxins content in grain (Yoshida *et al*., 2007). Mycorrhizal fungi are ubiquitous symbiotic microorganisms associated with plants which could have an important impact on plant interaction whit pathogenic fungi and insects (Pozo and Azcon-Aguilar, 2007). There are several studies in literature review that have demonstrated the potential of Arbuscular Mycorrhizal Fungi (AMF) for biological control and their impact on sustainable agriculture (Azcón-Aguilar and Barea, 1997; Hooker *et al*., 1994; Leyval *et al*., 2002; Vigo *et al.,* 2000). The plant growth promoting rhizobacteria (PGPR) include free living bacteria that colonize the roots of monocots and dicots and enhance plant growth by an exorbitance mechanisms (Nadeem *et al*., 2014; Vacheron *et al.,* 2013). The majority of PGPR systems induce the growth of plant which could lead to better defense mechanisms to control many pathogenic micro-organisms such as fungi, nematodes, bacteria and viruses organism (Vessey, 2003). Vermicomposts, which are produced by the fragmentation of organic wastes by earthworms, have a fine particulate structure and contain nutrients in forms such as nitrates, exchangeable phosphorus and soluble potassium, calcium, and magnesium that are readily taken up by plants (Arancon *et al*., 2005; Atiyeh *et al*., 2000; Orozco *et al*., 1996). The role of vermicompost in reducing some pathogenic fungi and disease incidence have been reported (Edwards *et al.,* 2004; Litterick *et al.,* 2004). Several classes of systemic acquired resistance genes have been reported to induce resistance to FHB in wheat. One group of genes referred to Pathogenesis-Related (PR) or defense response genes, encode proteins such as *β*-1,3-glucanases, chitinases, thaumatin-like proteins (tlps) and thionins whose expression often increase as part of the plant host defense response to pathogen attack (Linthorst and Van Loon, 1991). This study aimed to demonstrate the effect of different bio-fertilizers application on plant growth and some yield components in Fusarium infected wheat. To better understand the role of nutrition to induce systemic acquired resistance (SAR), the expression levels of some defense response genes against pathogenic fungi including *β-1,3-glucanase*, *oxalate oxidase* and *chitinase* genes were analyzed.

MATERIALS AND METHODS

Plant Culture

A wheat cultivar susceptible to head blight disease (cv. Tajan, Iran) was used in this study. Wheat seeds free of microbial contamination were cultivated in plastic pots (16.5×5.5 cm) containing sterilized (1 hour at 121°C) sandy-loam-clay soil (1:2:1) for one hours at 121°C. Chemical characteristics of the soil were analyzed before the experiment (Table1).

Fertilizer Treatments

This study was conducted on completely randomized design with four replications under greenhouse conditions. The bio-fertilizer application included control (Fusarium infected wheat without fertilizer), arbuscular mycorrhizal fungus (*Glomus intraradices)* (15% v/v), Nitroxin (primimg), Vermicompost (20% v/v), Nitroxin+Vermicompost (N+V), Mycorrhizal+Vermicompost (M+V) and Mycorrhizal+Nitroxin (M+N) were used.

Table 1. Physical and chemical characteristics of soil and vermicompost.

	Sand	Clay (%)	Salt	Zn	Fe (ppm)	K	P	N (%)	pH	EC (dSm^{-1})
Soil	41	32	27	4.8	2.2	185	12	6.3	7.1	1.8
Vermicompost						626	13	1.6	8.1	5.6

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Wheat seeds were planted 3 cm deep in the soil and then were covered with 1.5 cm of autoclaved vermiculite. Yield components such as 100 seeds weight, root length, spike length, root dry weight, and biological yield were measured 72 hpi.

Fungal Inoculums

Standard isolates of *F. graminearum* species was used as inoculums. Macroconidia was produced by re-culture of fungi on Carnation Leaf Agar (CLA) in sterile condition. The cultures were incubated in darkness at 25°C for one week and then the conidia were washed from culture surface and counted using a haemocytometer. The suspension concentration was adjusted to 5×10^5 spores mL^{-1}, and stored at -20°C until use. Inoculation was carried out by injecting between the palea and lemma of 10 central spikelets per each spike on different plantlets using 1 mL cell suspension at 10^5 cells mL^{-1} (Nemati and Navabpour, 2012) .The plants were grown in a night/day temperature of 18/24±5°C under greenhouse condition.

RNA Isolation and RT-PCR

Total RNAs from treated and control plants were isolated using GeneAll Kit (South Korea) and purified using RNA purification Kit (Promega, Cat. No.: AS1500). Total RNA was quantified using a Scandrop spectrophotometer (AnalytikaGena, Germany) and RNA quality was assessed by 1% agarose gel electrophoresis stained by ethidium bromide and photography by Geldocument (Vilber, France). First-strand cDNA was synthesized from total RNA using 2-Steps RT-PCR kit (Vivantis, Sinaclon Co. Cat. No.: RT5201) and then fifty to 100ng of total RNA was used to prepare double-strand cDAN:

Three genes including *β-1,3-glucanase* (Gene Bank TM accession number: DQ090946.1), *Oxalat Oxidase (*AJ556991) and *Chitinase* (AY437443.1) were used for expression analysis. *β-Tubolin* gene was used as housekeeping gene.

qRT-PCR

Reverse Transcriptase-quantitative Polymerase Chain Reaction (RT-qPCR) was carried out in Roter Gene (RG-3000 Coorbet Research) and PCR MasterMix for Syber Green Assays (Hot Tag EvaGreen, ROX, GeneAll, South Korea; Cat. No.: 16-100), according to the manufacturer's protocol. The amplifications were performed using the following concentration: 4 µL of SYBR Green PCR MasterMix , 1 µL of each oligo-nucleotide primer (final concentration 10 µM) and 1 µL of cDNA template in 30 µL reaction volume. Each gene amplification was prepared in triplicates and two biological repetitions were carried out. Triplicates were validated with technical error under 0.5 CT. The amplification condition was: 95°C for 10 minutes, 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. Melting curves analysis were performed after each reaction, to exclude non-specific amplifications, with the thermal cycle at 95°C for 15 seconds, 60°C for 15 seconds and 95°C for 15 seconds. The optimal baseline and threshold values were determined using automatic CT function available. Relative gene expression levels for the three replications were calculated using the REST method (Pfaffl, 2001)

Data Analyses

The significant difference was set at $P\leq 0.05$ and determined using the Least Significant Difference (LSD) multiple range tests. Data for plant height (cm), shoot dry weight (kg), root dry weight (kg), 100 seed weight, seed number, biologic yield, seed yield, spike length (cm) and root length (cm) were recorded for each treatment and subjected to statistical analysis (One-way ANOVA) using SAS software.

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RESULTS

The growth and yield compounds of infected wheat with *F. graminearum* as root length, plant height, shoot and root dry weight, weight and number of grains and biological yield parameters were significantly increased at the 1% probability level by all applied bio-fertilizers (Table 2).

Root Length and Plant Height

A significant difference among treatments for plant yield parameters was observed. Maximum plant height at 61 cm was recorded for combined M+V treatment whereas the lowest average plant height of 54 cm was obtained with mycorrhizal treatment (Table 2). The highest average root length of 15 cm was obtained with M+V and N+V biofertilizer combination (these treatments are listed statistically into one group), and the lowest average root length of 10 cm was found for mycorrhizal treatment (Figure 1).

Shoot and Root Dry Weight

According to the results of yield components assay, the highest average shoot dry weight of 5.7 g pot^{-1} was found in N+V treatment and the lowest average amount of 2.71 g pot^{-1} was record for mycorrhizal treatment. The results also showed that the highest average root dry weight of 2.14 g pot^{-1} was obtained for control (this treatment was listed statistically into one group with N+V treatment) and the lowest average root dry weight of 0.78 g pot^{-1} was recorded in N+M treatment (Figure 2). According to our observation, it seems that biological and organic fertilizers had no statistically significant effect on growth and development of Fusarium infected roots.

Grains Weight and Number

The effects of treatments on 100 grain weight showed a significant difference ($P< 0.05$) on 100 grain parameter in different treatments. The highest effect of bio-fertilizer on this parameter was found in the combined M+V application (2/44 g pot^{-1}), but there was no statistically significant difference in comparison with other applications. The lowest 100-grains weight was recorded in mycorrhizal treatment (1.68 g pot^{-1}). Data analysis showed the highest average seed yield (0.23 g pot^{-1}) in M+V treatment and the lowest average seed yield (0.03 g pot^{-1}) with mycorrhizal application (Table 3).

Table 2. Analysis of variance for dry weight, root dry weight, 100-seed weight, seed number, biological yield, seed yield, plant weight and spike length traits in wheat infected by F. oxysporum under different bio-fertilizers treatment.[a]

Treatment	Root length	Spike length	Plant height	Seed yield	Biologicl yield	Seed number	Seed weight
Control	12.5 ^{b}	8.96 ^{a}	54.66 ^{bc}	0.94 ^{d}	5.7 ^{ab}	5.5 ^{b}	1.75 ^{d}
My	10 ^{c}	8.33 ^{a}	54 ^{c}	0.03 ^{e}	2.7 ^{d}	3.83 ^{c}	1.68 ^{d}
N	12.4 ^{b}	8.66 ^{a}	55.66 ^{bc}	0.12 ^{c}	6.05 ^{a}	6 ^{b}	1.94 ^{c}
V	13 ^{b}	9.3 ^{a}	58.66 ^{ab}	0.18 ^{b}	5.2 ^{b}	7.5 ^{a}	2.25 ^{b}
N + V	15 ^{a}	8.76 ^{a}	57 ^{bc}	0.17 ^{b}	5.8 ^{ab}	7.25 ^{a}	2.18 ^{b}
M + V	15 ^{a}	9.41 ^{a}	61 ^{a}	0.23 ^{a}	5.83 ^{ab}	7.91 ^{a}	2.44 ^{a}
M + N	14.83 ^{a}	8.76 ^{a}	60 ^{a}	0.038 ^{e}	3.3 ^{c}	2.58 ^{d}	1.68^{d}

[a] Means in each column and for each treatment followed by similar letter(s) have not significantly different at 5% probability level.

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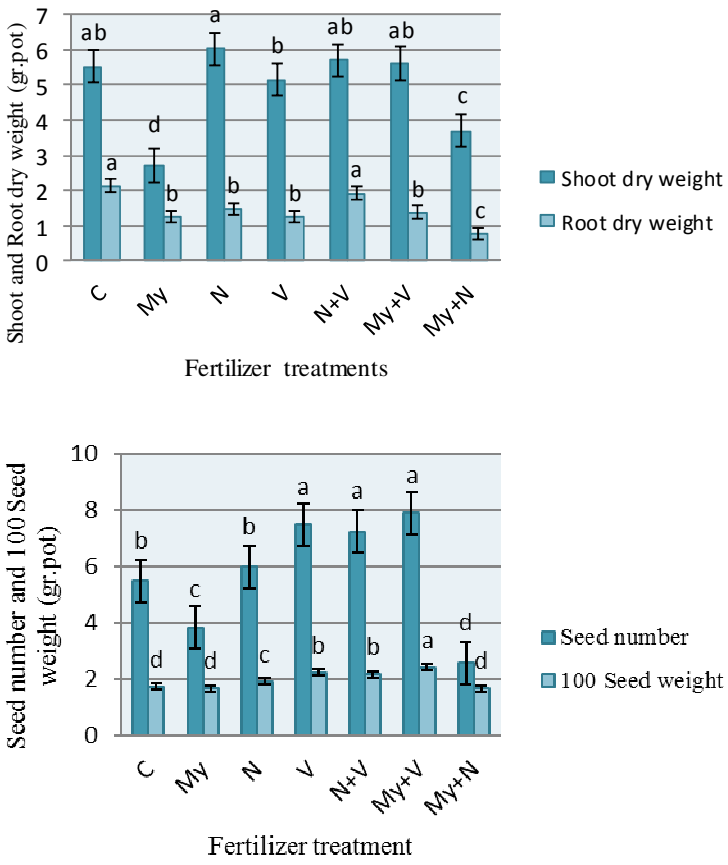

Figure 1. Effect of different bio-fertilizers on (a) shoot and root dry weight (b) seed number and 100 seed weight of wheat infected by *F. graminearum*. C: No fertilizer; M: Mycorrhizal ; N: Nitroxin, V: Vermicompost .

Table 3- Mean of shoot dry weight, root dry weight, 100-seed weight, seed number, biological yield, seed yield, plant weight and spike length under treatment of different bio-fertilizers in wheat infected by *F. graminearum.*

		Means of Square								
S.O.V	df	Shoot dry weight	Root dry weight	100-Seed weight	Seed No.	Biological yield	Seed yield	Plant weight	Spike length	Root length
Bio-fertilizers	6	4.56**	0.61**	0.27**	11.9**	5.46**	0.017**	21.7**	$0.43^{n.s}$	10.27**
Error	14	0.19	0.033	0.005	0.18	0.12	0.0014	6.1	0.32	0.73
Cv(%)		9	12.5	3.84	7.46	7.07	9.6	4.3	6.38	6.45

Biological Yield

The biological yield assay showed a significant effect (P< 0.05) of bio-fertilizer treatments (Table 2). The applied M+V and mycorrhiza treatments showed the highest (5.83 g pot^{-1}) and lowest (2.27 g pot^{-1}) effect on the biological yield characters, respectively (Table 2).

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Gene Expression Analysis

Gene expression level of *β-1,3-glucanase, oxalate oxidase* and *chitinase* genes were assayed in plant infected with *F. graminearum* species which were treated with different bio-fertilizers. The results of gene expression analysis showed that *β-1,3-glucanase* gene was over expressed in all treatments compared to the control. Transcript levels were elevated in V+M treatment compared with water-treated control plants. For *chitinase* and *oxalate oxidase* genes expression, there was no significant difference between inoculated plants treated with mycorrihza and the control. *Chitinase* gene expression in treated plants showed the lowest average value (Figure 3).

DISCUSSION

Wheat is the one of the most important grains in the food chain worldwide (Neo, 2011). *F. graminearum* is one causal agent of a destructive disease known as wheat scab in different parts of the world (Abedi-Tizaki *et al.,* 2013). In this study, we investigated the effect of some bio-fertilizers in single or combined forms on the growth, yield and systemic acquired resistance of fusarium infected wheat under greenhouse conditions. Head blight disease is one of the most destructive diseases of wheat, furthermore, it causes losses in quality and quantity of grains. Data presented in Table 2 demonstrate that the majority of bio-fertilizers application significantly increased ($P \leq 0.05$) grain yield, shoot and root length, root dry weight, plant height and biological mass when compared to the control. In general, the availability of water and essential nutrients, affect plant height through the number of nodes and internodes length (Morrison *et al.,* 1999). The effect of fertilizers as source of nutrition is clear. The role of mcorrhizal fungi has been defined as hyphal growth prevention or competing for nutrient and space (Limón and Codón, 2004). In this research, our data presented in Table 2 show that mycorrhizal application did not approximately increase all yield components compared to the control plants such that the lowest average parameters was determined in mycorrhizal treatment which could possibly be due to the fact that the presence of pathogenic fungi *F.*

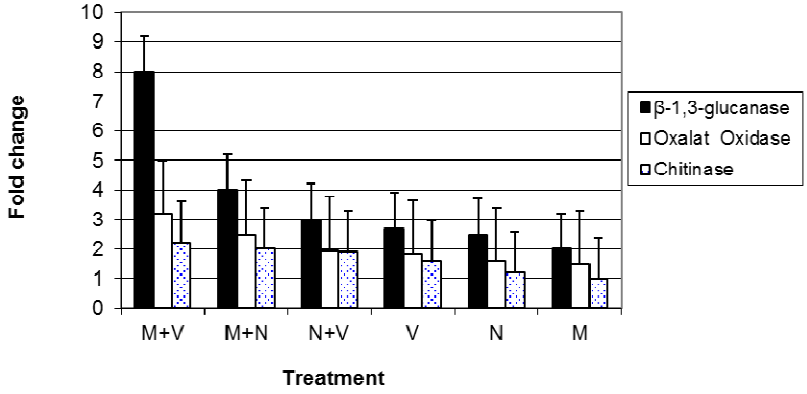

Figure 3. Quantitative real-time PCR analysis of of *β-1,3-glucanase, oxalat oxidase* and *chitinase* genes. The relative fold change of target gene transcripts was calculated using the comparative cycle threshold method Ratios are given as logarithmic values (base 2) of means of three independent experiments, means were calculated with REST. *β*-1,3-glucanase (black bar) shows higher expression than other tested genes (P-value 0.001, determined with REST). Gene expression analysis in infected wheat by *F. graminearum* treated with different bio-fertilizers.

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graminearum led to reduction of bioprotection of arbescular mycorhizal fungi on head blight disease development. These results are not fully comparable with the positive result of mycorrhizal application in rhizospher, because in this study, the locations of mycorrhizal (soil) and pathogenic fungi (aerial parts) agent were differed. Nitroxin bio-fertilizer containing a mix of nitrogen-fixing bacteria belong to Azotobacter and Azospirillum genus that cause an increase in growth and development of shoot and root of plants (Glick *et al.,* 2001). The biological fixation of the nitrogen by bio-fertilizers could have an important role in improvement and fertility of soil. Nitroxin is a biologic nitrogen fertilizer that contains *Azospirillum (Spirilaceae)* and *Azotobacter* (Vlassak *et al*., 1992). It has been found that Nitroxin applications lead to the highest impact on seed weight and biological yield of rapeseed plant (*Brassica napus* L.) (Azimzadeh and Azimzadeh, 2013). In the present study, increase in shoot dry weight with combined N+V treatment could be due to the beneficial effect of *Azotobacter* and *Azospirillum* bacteria on root growth. Application of *Paenibacillus polymyxa* (SQR21) as a bioorganic fertilizer for improving the biocontrol efficacy to Fusarium wilt disease of watermelon showed that the number of colony-forming units of *Fusarium oxysporum* in rhizospheric soil was significantly ($0.05 \leq P$) inhibited compared to the controls (Ling *et al.,* 2010). In this study, pathogenic agent and biofertilazer microorganisms did not have any interaction and positive response to disease could be due to nutrition and improvement of plant growth condition. Also, in the study of Ling *et al.* (2010), there was not any interaction between bacterial strain and pathogenic fungi, in agreement with our results.

Plant Prowth Promoting Rhizobacteria (PGPR) can affect the pathogens directly and indirectly and are able to elicit Systemic Acquired Resistance (SAR) in plants defense against different pathogenic agents (Kumar *et al.,* 2005). Increase of 100-grain weight could be due to the effect of organic fertilizers on seed yield through nutrient assimilation and seed filling. The highest significant increase in 100-grains has been reported in Isabgol medical plant with mycorrhiza application (Singh *et al*., 2003). Our observation showed that mycorrihzal fungi could not independently increase studied traits, but in combination with vermicompost had significantly influenced all growth parameters. Our results are in concordance with results of Norman and co-workers (2005) who showed that vermicompost had a positive effect on mycorrhizal symbiosis percentage and extension of external hyphae (Norman *et al.,* 2005). Also, the results showed that the highest average number of seeds per spike (7.91 g pot^{-1}) was recorded in the combined M+V treatment and the lowest average of 2.58 g pot^{-1} was obtained in the single mycorrhizal treatment (Table 2). The number of seeds is the most important yield component that is determined during the period from florets initiation to seed filling. In fusariose disease, spike is the most important site effect of disease which lead to losses of grain weight, so, in this study, we did not expected high increase in this trait due to fungal growth in panicle. For this reason, all traits related to grain have not significantly increased and, in some treatment, they have been decreased when compared to the control. To illustrate the effect of bio-fertilizer on disease development and toxin production, a chemical analysis by HPLC method to determine quality of different trichothecene toxins is necessary (Abedi-Tizaki and Sabbagh, 2013). To increase yield production (seed number and weight), adsorption of nitrogen at the flowering stage should be increased (Ruffo *et al*., 2003; Wiersma *et al.,* 1996). Other studies have shown that vermicompost application increases yield, improves soil biological properties, and also provides macronutrients in the soil (Norman *et al.,* 2005). Increase in biological yield of many higher plants

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treated with symbiotic mycorrhizal fungi has been reported (Treseder and Cross, 2006). The application of nitrogen with mycorrhizal fungi increased biological yield in wheat and barley (Behl *et al.,* 2012) while the application of mycorrhizal fungi with vermicompost increased the biological yield in sorghum (Cavender *et al*., 2003). Our data are in agreement with these observations. This study showed that vermicompost has no direct effect on mycorrhizal symbiosis percentage while it suggested that the effect of vermicompost was to provide nutrients for mycorrhizae development and growth of the host plant roots. Expression pattern of transcribed mRANs in susceptible varieties of wheat using microarray and SSR approaches has shown an up-regulation of defense-related genes occurring early during fungal stress (Bernardo *et al*., 2007). Recently, a set of cDNAs sequences as plant genes which confer resistant to triochothecen mycotoxines have been patented (Tumer *et al*., 2014). Change of these genes could be used as a biomarker for determining mycotoxines in different state of disease in wheat infected by *F. oxysporum,* but unfortunately, at this time, these sequences were not accessible and we were obligated to use defense related genes in wheat that have been reported against different pathogenic agents (Kong *et al.,* 2005; Abedi-tizaki and Sabbagh, 2013). Gene expression analysis in susceptible and resistant cultivars of wheat showed that the timing of defense response gene induction correlates with *F. graminearum* infection and their transcripts were accumulated as early as 6 to 12 hours post infection and peaked at 36 to 48 hpi (Pritsch *et al*., 2000). Based on these data, we analyzed the expression level of three defense response genes, 72 hpi in treated plantlets with different bio-fertilizers in single and mixed combination. Our data indicate that *ß-1,3 glucanase* gene was over-expressed compared to the other tested genes in all treatments. These results are in agreement with those of Nemati and co-worker who reported an increase in expression of *ß-1,3 glucanase* gene in FHB susceptible variety Sumi3 (Nemati and Navabpour, 2012). As shown in Figure 3, expression level of o*xalate oxidase* and *chitinase* did not show remarkable increase when compared to than *ß-1,3 glucanase* gene. Lower expression of o*xalate oxidase* gene as a key enzyme in antioxidant activity could indicate that this gene has no important role in SAR reaction. For *ß-1, 3 glucanase* gene, the highest expression level (8 fold change) was recorded for M+V treatment and the lowest was observed for mycorrhizal bio-fertilizer (2 fold change) application which confirmed the result of yield components where mycorrizal treatment showed low effect on studied traits (Table 2).

Chitinase and *ß-1,3 glucanase* genes have synergistic activity and simultaneous expression resulted in increased fungal resistance in infected tomato plants (Jongedijk *et al.,* 1995). In spite of low expression of *chitinase* gene in treated plants, according to accumulation role of chitinas and *ß*-1,3 glucanase we can conclude that chitinase could have synergistic activity with *ß*-1,3 glucanase to induced resistance.

Little information exists regarding the role of bio-fertilizers on induced resistance through soil improvement. In this work, we attempted to investigate the interactions of different bio-fertilizers in the rhizosphere and their effects on aerial parts by induced systemic acquired resistance.

Our data showed that all bio-fertilizers in single and combined forms are able to trigger systemic acquired resistance in Fusarium infected wheat. So, we can suggest biofertilizer combination for high yield components which could result in systemic resistance in infected wheat by *F. graminearum.* Applied M+V fertilizer was, however, the best combination to increase yield component and defense response genes expression. Expression level of *chitanase* gene was determined to be the lowest on all treatments, indicating that *ß-1,3 glucanase* and *oxalat oxidase* genes had a key role in

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SAR and their related pathogenesis proteins in resistance pathway.

CONCLUSIONS

Based on our results, we could conclude that the appropriate combination of effective bio-fertilizer is the mixture of vermicompost and mycorrhizal fungi. Therefore, combined application of these fertilizers could have more efficiency because of some positive interaction between their micro-organisms. Vermicompost improves the growth condition for mycorrhizal fungi. So, this could be proposed for use as an alternative bio-fertilizer in an application formula. However, this study was conducted in a greenhouse with autoclaved soil to reduce or eliminate fungal pathogens. Therefore, these results cannot be generalized for field conditions. Hence, it is recommended that the research be carried out under field conditions. However, heavy metal uptake by plants could increase by bio-fertilizers application. So, to elucidate toxicological activity of bio-fertilizers, especially bio-control fungus, preliminary tests for heavy metal accumulation assay in root of plant is necessary before recommending field application.

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کودهای بیولوژیک و القاء مقاومت سیستمیک در گندم آلوده به فوزاریوم

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چکیده

کودهای زیستی به عنوان یک جایگزین کودهای شیمیایی برای رشد و سلامت گیاه معرفی شده اند. هدف از این آزمایش کشت گلدانی، ارزیابی اثرات سه کود زیستی به صورت تنها و مخلوط شامل قارچ میکریز آربسکولار (*Glomus intraradices*)، نیتروکسین و ورمی کمپوست بر روی رشد، اجزاء عملکرد و بیان چندِ ژن پاسخ دفاعی در گیاه گندم آلوده به فوزاریوم بودPCR . در زمان واقعی برای تعیین سطح بیان ژن های بتا ۱-۳ گلوکاناز، اگزالات اکسیداز و کیتیناز انجام شد. استفاده از کود زیستی به طور قابل توجهی تمام پارامترهای مورد مطالعه به جز طول سنبله، در بوته های آلوده را افزایش داد. بالاترین میزان وزن خشک ساقه برای تیمار N + V و بالاترین ارتفاع بوته، تعداد دانه، وزن ۱۰۰ دانه و عملکرد بیولوژیک برای تیمارM + V مشاهده شد. استفاده از کود زیستی منجر به بالاترین سطح بیان در ژن بتا ۱-۳ گلوکاناز گردید .ژن کیتیناز پایین ترین سطح بیان را در تمام تیمارهای بکار رفته نشان داد. نتایج ما نشان می دهد که کاربرد ورمی کمپوست می تواند تحت کلنیزه کردن میکوریز را بهبود و روی توسعه هیف قارچ موثر باشد.

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