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Potential control of beans (*Phaseolus vulgaris* L.) wilt disease using growth regulators, bioagent, antioxidants and essential oils as foliar application under field conditions

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The efficacy of some fungicide alternatives as foliar spray was evaluated against bean wilt incidence under field conditions. The fungicide alternatives were indole acetic acid, gibberllic acid, *Trichoderma harzianum*, vitamins E and C, lemon grass, moringa leaf and thyme oils. The obtained results showed that the applied fungicide alternatives treatments could suppress the incidence of green bean wilt. In the light of the present study, a thought-provoking outcome of the following investigation was reached when the results clearly indicate that 100% suppression of the disease was reached when the bean plants were sprayed with a combination of indole acetic acid 40 ppm + gibberellic acid 40 ppm + *T. harzianum* 10^5 cfu/ml. Such an arrest in disease development decreased to 90.5 and 86.4% when the infected plants were treated with indole acetic acid 20 ppm + gibberellic acid 20 ppm + *T. harzianum* 10^5 cfu/ml and *T. harzianum* was combined with gibberellic acid 40 ppm, respectively. It could be hypothetically suggested that combined treatments between growth regulators with the bioagent as foliar spraying might be used practically for controlling such soilborne diseases replacing fungicides treatments.

Key words: Bean, biocontrol, foliar formulations, fungicide alternatives, wilt disease control.

INTRODUCTION

The oldest known beans have been known since earliest historic times found in the 5th dynasty tombs where they are mentioned in one of Ramses II's paeans. In medicine, ancient Egyptians used beans in remedies against constipation, as a remedy for a sick tongue, treatment for male urinary complaints and when women ate beans on an empty stomach as a birth control method. Common bean (*Phaseolus vulgaris* L.) is one of the most widely cultivated food legume species in the world (Baudoin et al., 2001) for local consumption and exportation purposes and is a worldwide food-secure and nutritious worldwide crop to people of all income categories (Pachico, 1993)

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especially to the poor as a source of dietary protein because animal protein such as meats and fish is often rare or completely absent from their diets (Beebe et al., 2013). Bean is attacked by certain pathogenic fungi causing wilt, root-rot and leaf spot diseases which seriously affected both plant stand and yield production. These organisms can occur individually throughout the growing season.

Bean plants influenced genuinely by wilt which is broad disease in the globe also is frequently viewed as concerning illustration of major issue of bean production, diminishing as well as decreasing both yield and quality (El-Mougy, 2001; El-Mougy et al., 2007). Yield losses caused as a result of bean wilt disease are more notable in developing countries due to higher abiotic stress were recorded. The main pathogen responsible for wilt incidence of bean was reported to be *Fusarium oxysporum* f. sp. *phaseoli* (Burnchara and Camacho, 2000; El-Mougy, 2001).

The control of bean wilt depends on chemical control, biological control, resistant cultivars and control by cultural practices. There is therefore a need to develop tools and procedures that are simple, fast and accurate for the quantification of pathogen populations, particularly, *Fusarium* species. In order to overcome such hazardous control strategies, scientists, researchers from all over the world paid more attention towards the development of alternative methods which are, by definition, safe in the environment, non-toxic to humans and animals and are rapidly biodegradable. One of such strategy is the use of biocontrol agents (BCAs) to control fungal plant diseases. Among the BCAs, species of the genus *Trichoderma* is most promising and effective biocontrol agent. *Trichoderma* as antagonist controlling wide range of microbes (Chet et al., 1977), and their mechanism of mycoparasitism is much more complex, involves nutrient competition, hyperparasitism, antibiosis, space and cell wall degrading enzymes.

A successful disease-control program could involve just a single practice, but the long term reduction of disease losses generally requires the application of several control measures. The best way to ensure success of a disease-management program is to use integrated disease-control measures (Dik et al., 2002). Generally, IPM is regarded as the use of environmentally safe practices to reduce the disease incidence and development or use of multiple control tactics integrated into a single pest control strategy (Zinkernagel et al., 2002). For example, different natural products, that is, biocontrol agents, plant extracts, essential oils, antioxidant, growth regulators and natural compounds were used as an IPM program which takes a somewhat different approach in plant disease control (Bindu and Kumar, 2009; Li et al., 2009; Sharma et al., 2012). A considerable concern in discovering plant-derived anti-microbial agents arised recently (Sagdic et al., 2003) for alternative application in the strategy of preventing bacterial and fungal growth (Lanciotti et al., 2004). Volatile compounds and essential oils used as alternatives for anti-bacterial and anti-fungal treatments (Jenny, 2000; Michael, 2000). Furthermore, Juglal et al. (2002) studied the effectiveness of nine essential oils to control the growth of mycotoxins producing moulds and observed that clove, cinnamon and oregano were able to prevent the growth of *Aspergillus parasiticus* and *Fusarium moniliforme.* Benkeblia (2004) observed an inhibitory effect of onion essential oil at different concentrations on the growth of *F. oxysporum, Aspergillus niger* and *Penicillium cyclopinum.*

In Egypt, the farmers replant the missed holes on the same field. This practice could leads to a high population of the pathogens, causing serious losses which could reach up to 12% (Anonymous, 2012). The Egyptian farmers followed unwise and intensive use of fungicides for disease management. This strategy proved to be an unsatisfactory solution for controlling wilt disease in Egypt. Research over the last few decades for alternative substances with fungicidal properties has revealed that a potentially useful component of integrated disease management (IDM) programmes against foliar fungal pathogens can be spray applications (Fallik et al., 1997; Mann et al., 2004; Mitchell and Walters, 2004). Therefore, recently, great attention has been diverted to the use of fungicide alternatives for suppressing plant diseases (El-Mougy, 1995, 2001, 2009; Siddiqui et al., 2001; El-Gamal et al., 2003; El-Mougy et al., 2004, 2007; Abd-Alla et al., 2009; Abdel-Kader, 1997; Abdel-Kader et al., 2011, 2012; Abdel-Kader and El-Mougy, 2014) to decrease human and animal health risk concerns. Increased use of foliar fungicides has prompted us to explore potential impacts of this change in production practices and determine if foliar non-fungicide use can be justified for use of other than disease management.

In the present study, an investigation control method to disease development and to reduce the yield losses caused by wilt using non-fungicidal methods of treatment was performed. In this concern, we aimed to determine the efficacy of some fungicide alternatives growth regulators e.g. indole acetic acid and gibberellic acid individually or combined with the bioagent *T. harzianum*, lemon grass, moringa leaf and thyme (essential oils) or vitamins C and E (antioxidants) as foliar spray against bean wilt incidence under field conditions.

MATERIALS AND METHODS

Tested materials

Green bean (*P. vulgaris*) seeds cv. Giza 3 obtained from Vegetables Crop Research Department, Agricultural Research Centre, Giza, Egypt. The growth regulators indole acetic acid (IAA) and gibberellic acid (GA) and antioxidants (Vitamins E and C) were purchased from Al-Gamhoria Company Ltd. for chemicals and medicinal instruments, Cairo, Egypt. Commercial essential oils of lemon grass (that is, citral, geraniol, borneol and citronellol), moringa leaf (that is, significant source of B vitamins, vitamin C, provitamin A as beta-carotene, vitamin K, manganese, and protein, among other essential nutrients) and thyme (that is, thymol, carvacrol, geraniol, thymol methyl ether, a-pinene) were used in the present work. Essential oils were purchased from Chemical Industrial Development Company (CID), Egypt. The bioagent, used in this study is candidate antagonistic isolate of *T. harzianum*, obtained from Culture Collection Unit, Department of Plant Pathology, National Research Center, Cairo, Dokki, Egypt. This antagonist was isolated from the rhizosphere of various healthy and wilt or root rot infected leguminous crops, grown in the Delta and Middle Egypt regions, and proved its high antagonistic ability against different pathogens during previous work at the same department.

Agents

Field experiment was conducted at Researches and Production Station of National Research Centre at Nubaria region, Beheira Governorate, Egypt during 2014 and 2015 two winter growing seasons to evaluate the efficacy of some foliar spray treatments for controlling green bean wilt disease incidence. This field is well known by the authors throughout previous studies as naturally heavily infested with soilborne wilt pathogens. Also, it was observed that soil infestation with wilt pathogen is not equally distributed in all over this field, but it seems to be characterized with semi-homogeneous distribution with wilt pathogens.

In the present work, the applied treatments were designed as follows: Indole Acetic Acid 10 ppm; Indole Acetic Acid 20 ppm; Indole Acetic Acid 40 ppm; Gibberellic Acid 10 ppm; Gibberellic Acid 20 ppm; Gibberellic Acid 40 ppm; Indole Acetic Acid 10 ppm + *T. harzianum* (10^5 cfu/mL^{-1}); Indole Acetic Acid 20 ppm + *T. harzianum* (10^5 cfu/mL^{-1}); Indole Acetic Acid 40 ppm + *T. harzianum* (10^5 cfu/mL^{-1}); Gibberellic Acid 10 ppm + *T. harzianum* (10^5 cfu/mL^{-1}); Gibberellic Acid 20 ppm + *T. harzianum* (10^5 cfu/mL^{-1}); Gibberellic acid 40 ppm + *T. harzianum* (10^5 cfu/mL^{-1}); Indole Acetic Acid 10 ppm + Gibberellic Acid 10 ppm + *T. harzianum* (10^5 cfu/mL^{-1}); Indole Acetic Acid 20 ppm + Gibberellic Acid 20 ppm + *T. harzianum* (10^5 cfu/mL^{-1}); Indole Acetic Acid 40 ppm + Gibberellic Acid 40 + *T. harzianum* (10^5 cfu/mL^{-1}); *T. harzianum* (10^5 cfu/mL); Vitamin E+ lemon grass oil (2% v:v); Vitamin E + Moringa leaf oil (2% v:v); Vitamin E + thyme oil (2% v:v); Vitamin C + lemon grass oil (2% v:v); Vitamin C + Moringa leaf oil (2% v:v); Vitamin C + thyme oil (2% v:v); Untreated control

Field study

Certain weights or volumes of tested materials were added to 20 L water to obtain the proposed concentration used (Abdel-Kader et al., 2011). The inoculum of the bioagent *T. harzianum* was used as spore suspension at the rate of 10^5 cfu/mL^{-1} (Abdel-Kader et al., 2012).

Stocks solutions at high concentrations (ppm) of tested growth regulators IAA and GA were prepared by dissolving in sterilized distilled water, and then different volumes of the prepared solution were added to 20 L water to obtain the proposed concentrations. The tested essential oils were added individually to 20 L water to obtain the proposed concentration of 2%. A few drops of the emulsifier Tween 20 (Sigma Co.) were added to the essential oil volumes to obtain an emulsion feature. As for antioxidant (vitamin C and vitamin E), certain weight of each were dissolved in 20 L water to obtain the proposed concentration of 2%.

All foliar spray treatment were applied twice, the first at the emerged stage of bean plants (at two true leaves age) and the second after 15 days interval.

The test field comprised of plots (4×4 m), each comprised of 5 rows (5 holes/1 m and 20 holes/row). Green bean seeds Giza, 3 *cv*. were sown (at 15 September 2014 and 2015) in all treatments at the rate of one seed/hole. Furthermore, the emerged bean seedlings were thinned to obtain a uniform number of 80 seedling/plot, in order to neglect the incidence of pre-emergence damping-off infection of bean seeds from present work calculation. Concerning illustration five replicates for every specific treatment and in addition untreated plots were taken in consideration. The traditional agricultural practices, that is, soil plowing, fertilization, irrigation, etc., were followed at all experimental plots. Monitoring and scouting for disease incidence in all cultivated plots were preformed weekly (El-Mougy, 2001). Then, bean plants showing wilt symptoms were recorded. The isolated wilt incident was identified as *Fusarium oxysporum*. Average percent of wilt disease infection was recorded 15 days after each applied spray and the average accumulated disease incidence was calculated at the flowering stage (60 days old) of plant growth.

The field experiments were carried out for two successive winter growing seasons 2014 and 2015 at the same field. The average percent of wilt incidence for the two growing seasons was calculated and recorded as mean disease incidence. Disease incidence and reduction were calculated as following equations:

Disease incidence = C-T/T ×100

where C is the number of diseased plants and T is the total number of plants.

Disease reduction= D – D_1 / D × 100

where D is the number of diseased plants in control and D is the number of diseased plants in treatment.

Statistical analysis

The obtained data of field experiments were set up in Completely Randomized Design (CRD). The data collected were analyzed by MSTAT-C program (MSTAT-C, 1988). The means differences were compared by Least Significant Difference test (LSD) at 5% level of significance. The statistical analysis procedures were kindly carried out by Statistical Consulting Office, National Research centre, Egypt.

RESULTS

Evaluation of some fungicide alternatives against wilt incidence was carried out under field conditions for two successive growing seasons.

Evaluation of growth regulators

Generally, we observed that the results of the disease control under the effect of Indole Acetic Acid and Gibberellic acid when each was used independently or in combination with the biocontrol agent, *T. harzianum* depended on their used concentration (Table 1). A dose-

Table 1. Effect of growth regulators and antioxidants combined with bioagent or essential oils against wilt disease incidence of green bean under field conditions during two successive growing seasons.

Foliar spray treatment	Wilt incidence (%)*	Disease reduction (%)
Indole Acetic Acid 10 ppm	20.0	23.9
Indole Acetic Acid 20 ppm	16.2	38.4
Indole Acetic Acid 40 ppm	12.5	52.4
Indole Acetic Acid 10 ppm + *T. harzianum*	11.2	57.4
Indole Acetic Acid 20 ppm + *T. harzianum*	10.0	61.9
Indole Acetic Acid 40 ppm + *T. harzianum*	7.5	71.4
Gibberellic acid 10 ppm	18.7	28.8
Gibberellic acid 20 ppm	16.2	38.4
Gibberellic acid 40 ppm	13.7	47.9
Gibberellic acid 10 ppm + *T. harzianum*	11.2	57.4
Gibberellic acid20 ppm + *T. harzianum*	8.7	66.9
Gibberellic acid40 ppm + *T. harzianum*	6.2	76.4
Indole Acetic Acid 10 ppm+Gibberellic acid 10 ppm+*T. harzianum*	5.0	80.9
Indole Acetic Acid 20 ppm+Gibberellic acid 20 ppm+*T. harzianum*	2.5	90.4
Indole Acetic Acid 40 ppm+Gibberellic acid 40 ppm+*T. harzianum*	0.0	100
T. harzianum	7.5	71.4
Vitamin E + lemon grass oil	10.0	61.9
Vitamin E + Moringa oil	15.0	42.9
Vitamin E + thyme oil	10.0	61.9
Vitamin C + lemon grass oil	7.5	71.4
Vitamin C + Moringa oil	13.7	47.9
Vitamin C + thyme oil	7.5	71.4
Untreated control	26.3	-
LSD at 0.5%	2.8	-

*Wilt incidence was recorded as the mean percent of disease incidence at the two growing seasons and calculated relatively to the number of emerged bean seedlings= 80.

response experiment was conducted to determine the level of wilt incidence and reduction in the percentage of the disease to obtain a glimpse of a potential control mechanism.

The data revealed that increasing concentration of applied growth regulators showed parallel reduction in disease incidence. Moreover, the growth regulator indole acetic acid was more effective against disease incidence than gibberellic acid. The recorded reduction in wilt incidence ranged between 23.9 and 52.4% for indole acetic acid, meanwhile this range was from 28.8 to 47.9% for gibberellic acid at similar concentrations of 10, 20 and 40 ppm.

Consequently, our observation indicates that the wilt incidence percentage decreased from 23.9 to 38.4% and finally to 52.4% when indole acetic acid was applied while the recorded numbers under the effect of gibberellic acid recorded 28.8, 38.4 and 47.9%.

This indicates that the indole acetic acid when applied at a concentration of 10 and 40 ppm was more effective than gibberellic acid by 4.9 and 4.5%, respectively. At the concentration of 20 ppm, both agents had the same effect.

Evaluation of the bioagent *T. harzianum*

T. harzianum when applied during the experimental period at a concentration of 10^5 cfu/mL^{-1} caused a fall by 7.5% in the wilt incidence and 71.4% in disease reduction which represents a rise in the control of the wilt disease incidence by 28.5%.

Evaluation of combine effect of growth regulators and the biocontrol agent *T. harzianum*

The use of the bioagent *T. harzianum* in a combination with indole acetic acid and gibberellic acid increased the efficacy of the growth regulators against wilt incidence.
A combination of *T. harzianum* with indole acetic acid enhanced the suppressive effect of the growth regulator on the disease with an increase reduction in disease

incidence from (23.9 to 57.4%), (38.4 to 61.9%) and (52.4 to 71.4%) at concentrations of 10, 20 and 40 ppm, respectively.

Similarly, when *T. harzianum* was combined with gibberellic acid, the suppressive effect of this combination was more evident when the gibberellic acid was used at concentrations of 10, 20 and 40 ppm to cause increase reduction in the disease incidence from 28.8 to 57.9%, 38.4 to 66.9% and 47.9 to 76.4% which could be estimated as 29.1, 28.5 and 28.5%, respectively.

The impact of the three agents when combined together as a single treatment protocol was highly effective when the experiment recorded that the reduction percentage in the disease recorded 80.9 and 90.4% when indole acetic acid and gibberellic acid were used at a concentration of 10 and 20 ppm with 10^5 cfu/mL^{-1} *T. harzianum.* This propitious effect reached its climax when the plants were treated with indole acetic acid 40 ppm + gibberellic acid 40 ppm+ *T. harzianum*10^5 cfu/mL^{-1} to protect the plants from wilt disease resulting in 0% infection (that is, a complete suppression in the incidence of green bean wilt).

Evaluation of the use of essential oils and antioxidants

The obtained results showed that the applied treatments of vitamin C combined with the essential oils of Lemongrass, Moringa leaf and thyme had superior effect on disease incidence recorded as 71.4, 47.9 and 71.4% comparing with vitamin E plus the same essential oils which recorded 61.9, 42.9 and 61.9%, respectively.

Generally, the presented data in Table 1 showed varied numbers of infected bean plants when treated with different treatments. These results could deduced that a percent of 15 and 13.7 infected plants were recorded in Vitamin E + Moringa leaf oil; Vitamin C + Moringa leaf oil treatments, meanwhile, 10.0 and 7.5% infected plants were recorded in both Vitamin E + lemongrass oil and Vitamin C + lemongrass oil treatments, in respective order. Thyme oil showed superior effect for reducing disease incidence when combined with Vitamin C that 7.5% infected plants were recorded when compared with 10.0% infected plants when combined with Vitamin E. Furthermore, it is interesting to note that wilt incidence was reduced by combining the growth regulators and bioagent *T. harzianum.* Respectively, indole acetic acid at 10, 20 and 40 ppm and gibberlic acid at 10, 20 and 40 ppm showed that 20, 16.2, 12.5% and 18.7, 16.2, 13.7% infected plants. Meanwhile, adding the bioagent *T. harzianum* reduced the infected plants to be 11.2, 10.0, 7.5% and 11.2, 8.7, and 6.2% in IAA and GA raising concentrations, in respective order. Combined treatments of IAA 10 ppm + GA 10 ppm + *T. harzianum* and IAA 20 ppm + GA 20 ppm + *T. harzianum* showed that 5 and 2.5% plants got infected out of 80 grown plants compared to 26.3% (21 plants) in untreated control. However, none of the plants have been infected by wilt disease (0%) when plants were treated with IAA 40 ppm + GA 40 ppm+ *T. harzianum*.

DISCUSSION

The need to increase food production by at least 70% in order to match the population increase is highlighted by the United Nations Food and Agriculture Organization. The fact that nearly 20% of the global harvest is lost due to plant diseases (Chakraborty and Newton, 2011) could make this demand more daunting. Chemical control using the application of fungicides could be one of the most efficient ways to combat these diseases. Furthermore, pathogens can develop resistance against the used fungicides which then the higher dose of chemicals are required.

In addition, the environmental and health concerns are associated with the potentially toxic of chemicals which is applied in fields. Therefore, the safer and more sustainable methods of crop protection are demand. A novel area of research in the war against pathogens focuses on enhancing the plant's defense system. If a plant can fight off an infection on its own, we can reduce the amount of pesticides needed. Similar to how children are vaccinated to protect against future diseases, plant pathologists are using the same methodology to enhance plants resistance against pathogens, with the goal of strengthening their defenses mechanism against invaders. For more explanation, the principle of "defense priming" terminology is very similar to how we develop vaccines to treat human diseases. A vaccine works by acting as a pathogen impostor. It tricks the immune system into thinking it is being attacked, which stimulates defense responses, such as the production of antibodies. This procedure creates a defense memory which could allow the immune system to remember a certain pathogen when the body is attacked in the future. It can then respond quickly referring to its primed memory from the vaccine. Furthermore, the plants prepared themselves to be ready for the enemy attacks. The treated plants with induce resistant agents display enhanced tolerance to pathogen infection, which is often characterized by fewer disease symptoms and reduction in pathogen populations within the plant. Although in commercial agriculture, primed plants have not yet been applied on large-scale, scientists are actively conducting their researches on the use of defense priming theory against bacteria, viruses and fungi under both greenhouse and field trials for plant protection (http://phys.org/news/2015-07-pathogens-immune.html#jCp).

This method which cause the induce defense systems could be considered as a safe and effective way to protect some of the global harvest currently lost due to diseases. The applications of fungicides have potential consequences, not only from the economic perspective, but from the biological impact on fungal populations and to have adverse environmental effects causing health hazards to humans and other non-target organism, including beneficial life forms. Increased use of foliar fungicides has prompted us to explore potential foliar use of others than fungicide use and disease management. This investigation focuses on crop disease management by using natural compounds derived from plants, marine organisms and microbes to represent an ecologically friendly approach for plant diseases control as an alternative to the chemical fungicides with no side effects on humans and animals. We evaluated some fungicide alternatives against wilt incidence under field conditions for two successive growing seasons. Different treatments of growth regulators alone or combined with the bioagent *T. harzianum* as well as antioxidants individually or combined with essential oils were applied as foliar spray on emerged green bean seedlings. The present study has demonstrated that all treatments tested, some growth regulators, essential oils and antioxidants have antifungal potential activities and could be useful when integrated with bioagents against green bean fungal pathogens.

Hypothetically, we visualize the mechanisms of action of the used agents in our investigation in controlling wilt of beans (*P. vulgaris* L.) as follows: (i) direct competition with the target organism; (ii) antibiosis; (iii) predation or parasitism of the target organism; (iv) induced resistance of the host plant; and (v) inactivation of the enzymes produced by the pathogen. These five intermingling mechanisms were seen when *T. harzianum* was used as our biocontrol agent. Recently, biological control of plant pathogens has received great attention as a promising supplement or alternative to chemical control and an attractive proposition to decrease heavy dependence of modern agriculture on costly chemical fungicides, which not only cause environmental pollution but also lead to the development of resistant strains. Commercial biopesticides are now available, and most of which are based on the free living fungi common in soil, associated with plant roots and root ecosystems, fungal genus *Trichoderma* (Woo et al., 2006) a genus under Deuteromycotina. They are non-plant pathogenic and have gained high importance as the most important and efficient biological control agents against plant pathogens (Lorito et al., 1998; Bokhari and Perveen, 2012). This could be attributed to their high reproductive capacity, ability to survive under unfavourable conditions, capacity to colonize the rhizosphere, strong antagonism against the pathogenic fungi and efficiency in promoting plant growth, defense mechanisms (Hanson and Howell, 2004; Segarra et al., 2007; Tucci et al., 2011; Reglinski et al., 2012) and to control soil borne fungal pathogens (Monte, 2001). *Trichoderma* spp. are potent mycoparasites attacking and parasitizing plant pathogens and also similarly to those of mycorrhizal fungi, whereas they are able to colonize plant roots and producing compounds that stimulate growth and plant defense mechanisms such as alkyl pyrones, isonitriles, polyketides, peptaibols, dikeyopiperazines, sesquiterpenes, and steroids which finally leads to induced systemic resistance (ISR) in the entire plant (Howell, 1998, 2003). An increase in peroxidase activity considered as indicator of plant response (often associated with the production of fungitoxic compounds), an increase in chitinase activity, and the deposition of callose-enriched wall appositions on the inner surface of cell walls. *T. harzianum* T019 indicates a certain impact on the resistance level for bean plants against *R. solani*. This strain induces the outflow from claiming plant defense-related genes and produces a larger amount of ergosterol, demonstrating its capacity for development in a higher rate in the soil, which might demonstrate its impacts around plant development and defense in the presence of pathogens (McLean et al., 2004; Mayo et al., 2015). Moreover, Abdel-Kader (1997) reported that *T. harzianum* introduced to the soil was able to reduce root rot incidence of bean plants significantly more than the fungicide Tolclofos methyl (Rizolex-T). The present work demonstrate that the use of the *T. harzianum* increased the effectively action of growth regulators against wilt incidence. Similarly in many countries, several workers stated that successful suppression of various plant diseases was achieved by using antagonistic microorganisms as biological controls application (Sivan and Chet, 1986; Sivan et al., 1987; El-Mougy, 2001; Whipps and Lumsden, 2001). These properties of *T. harzianum* clearly explains its ability to produce the highest degree of plant protection when these properties act in synergism with the properties of growth promoters as seen in our results (Indole Acetic Acid 40 ppm + Gibberellic acid 40 ppm + *T. harzianum* 10^5 cfu/mL^{-1}; Indole Acetic Acid 20 ppm + Gibberellic acid 20 ppm + *T. harzianum* 10^5 cfu/mL^{-1} and *T. harzianum* was combined with gibberellic acid 40 ppm, respectively).

When we used the growth promoters, the mechanisms of action of Indole Acetic Acid 40 ppm + Gibberellic acid in controlling wilt of beans (*P. vulgaris* L.) was attributed to a single distinct mechanism: induced resistance of the host plant. Recent studies on plant-pathogen interactions identify the major naturally occurring auxin (indole-3-acetic acid (IAA)) as a key character in pathogenesis and plant defense (Fu and Wang, 2011). Gibberellins (GA) as well as other plant growth hormones (auxins, cytokinins, abscisic acid, and brassinosteroids) regulating host defense responses triggered by the SA-JA-ET signaling systems and so they may be involved in plant immune expression (Asselbergh et al., 2008; De Vleesschauwer et al., 2010, 2012, 2013; Cao et al., 2011; Pieterse et al., 2012; Naseem et al., 2012; Naseem and Dandekar, 2012; Riemann et al., 2013; De Bruyne et al., 2014).

Plant immune responses have been shown by gibberellins through regulating and modulating JA and SA signaling systems (Navarro et al., 2006; Yang et al., 2008; De Bruyne et al., 2014; Qi et al., 2014). It induces resistance against different fungal and bacterial pathogens (Yang et al., 2008; De Vleesschauwer et al., 2012; Qin et al., 2013). Also, enhancing systemic acquired resistance against pathogens has been shown by GA (Xia et al., 2010) which act as central suppressors of signals causing degradation of DELLA proteins (a class of nuclear growth-repressing proteins) (Navarro et al., 2008). A highly specific blend of these ‘defense hormones’, produced by infected plants with the exact combination seemingly depending on the pathogen’s lifestyle. Increasing concentrations of GA for bean plants treatment enhanced their resistance in a concentration-dependent manner by regulating the plant immunity against *Pythium graminicola* (De Vleesschauwer et al., 2012).

On the other hand, the essential oils of Lemon grass, Moringa leaf and thyme induced their effect on the wilt as a dual mechanism: (i) direct competition with the target organism; and (v) inactivation of the enzymes produced by the pathogen. Various plant essential oils have been reported to have antifungal activities (Dabur et al*.,* 2007; Bindu and Kumar, 2009). Previous suggestion that plant oils proved their fungitoxic effect which may provide a renewable source of effective antifungal agents either *in vitro* or *in vivo* investigations (Ganesan et al., 2015) and to have the potential to replace the synthetic fungicides in the management of root fungi of fruit and vegetables. Generally, several microorganisms including many plants and human pathogenic fungi affected potentially by plant-derived essential oils and extracts which are considered at the same time as nontoxic compounds to plants (Tabassum and Vidyasagar, 2013).

The components of thyme essential oil reaches to 56 compound of which *p*-cymene (8.41%), γ-terpinene (30.90%) and thymol (47.59%). Geraniol, linalool, gamma-terpineol, carvacrol, thymol and *trans*-thujan-4-ol/terpinen-4-ol were the major constituents (Amiri, 2012). Lemon grass [*Cymbopogon flexuosus* (Steud.) Wats, (syn. *Andropogon nardus* var. *flexuosus* Hack; *A. flexuosus* Nees)] is one of the most worldwide grown essential oil plants which have antifungal and antibacterial properties (Zheljazkov et al., 2011) due to its major constituents caryophyllene oxide, *t*-caryophyllene, geranyl acetate, (*E*)-citral (geranial), (*Z*)-citral (neral), and trans-geraniol, citronellyl acetate, citronellol, geraniol and limonene.

The capacity of antioxidant, antimicrobial activity of the essential oil and hydrocarbons of *Moringa oleifera* (*Moringaceae*) was attributed to its chemical constants, thymol, (*E*)-phytol, hexacosane, pentacosane, heptacosane, hexacosane, pentacosane, and heptacosane. Hexanoic acid, acetic acid, nonacosane, 1, 2, 4-trimethyl-benzene, heptacosane, nonacosane, heptacosane, and pentacosane (1.0 to 6.3%) were among the most abundant constants in the essential oil worldwide (Marrufo et al., 2013; Leone et al., 2015). Moringa contains seven times more vitamin C than oranges. Moringa is a storehouse of vitamin E. The moringa leaves also provide significant amount of antioxidants. Antioxidants as vital to raise host plant resistance, triggering innnate immunity in plants to refine and promote methods of defense to the plant against the pathogens (Madukwe et al*.,* 2013).

When vitamins C and E were used in our endeavor, we kept in mind the fact that, there is no much research yet in this area. The newest research is suggesting and confirming the suggestions that vitamins serve healing purposes for the plants that produce them. Our investigation with vitamins C and E is beginning to show promise. Some plants have shown increased growth and ability to fight off bacterial disease with Vitamin C added to the soil or water (Last et al., 1997; Burkey, 2003; Smirnoff et al., 2007; Li et al., 2009). The antioxidants’ vitamins C and E play an important role in protecting cells and neutralizing free radicals during infection in addition to crucial roles in defense (DePinto and De Gara, 2004; Sharma et al., 2012).

Conclusion

Disease management strategy research should be largely focused on identifying crop and soil management approaches that have an immediate impact on managing root diseases. It is vital to raise host resistance, to refine and promote methods for the sustainable control of bean wilt simultaneously with the study of pathogenic variation. The information generated will be used as a basis for the development of improved crop management methodologies for sustainable production of beans by resource-poor, smallholder farmers. In this context, the present work emphasizes the importance of *Trichoderma* as a biological control agent and its application towards management of plant diseases. The present work also revealed a significant reduction in wilt incidence of green bean under field trials using integrated treatment application of growth regulators, essential oils or antioxidants with the bioagent *T. harzianum*. It may be concluded that such treatments are considered applicable, safe and cost-effective method for controlling such root diseases. So, induced resistance needs to find its way into plant diseases protection strategy and crop management programmes.

Conflict of Interests

The authors have not declared any conflict of interests.

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