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EFFECT OF SOME OF SAFE WAYS TO CONTROL WEB BLIGHT DISEASE THAT CAUSED BY RHIZOCTONIA SOLANI ON MUNG BEAN.

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Abstract
Web blight that infect mung bean has been known as the most common disease that reduce the production and quality this plant. To reduce the utilization of pesticides. Therefore, many strategies were used to manage plant diseases. In this study we used different treatments as nutrients supplement and bioagents. Results showed that the incidence of disease reduced by 62% and 91.3% with the formulation of adding Sulphur fertilizer and P. fluorescens in two different time of assessment. Although, disease severity reduced by 35.5% with same treatment this not statically differences. In addition, chlorophyll increased as well with P. fluorescens and Sulphur fertilizer by 84.2% and 82.8 % respectively in comparison with negative control. Number and weight of pods and plant high increased by 65.4%, 49.5%, and 86.55% respectively that with formulation of Sulphur fertilizer and P. fluorescens. While wet and dry weight of each of root and plant vegetative was increased with Sulphur fertilizer by 75.5%, 73.4%, 89.9%, and 82.2% respectively.
Key words: (web blight, mung bean, Sulphur fertilizer, T. harzianum, P. fluorescens).

Introduction
Mung bean is one of the legume crops that cultivated in three different seasons. So, there are many beneficial of it, seed of mung bean having around 24% protein, fiber, antioxidant and phytonutrient (Itoh et al; 2016). This crop can be induce to different disease, web blight (Vigna radiata L.) caused by Rhizoctonia solani (Teleomorph: Thanatephorus cucumeris (Frank) Donk) is an economically important disease which reduces 33 to 40 % grain yield at different level of disease severity and in different varieties of mung bean (Singh et al., 2012 and Gupta et al., 2010). This is due to the effect of disease on leaf area and seed quality then reduce the value of it. This disease can be spread by different way, it can spread between plant through mycelial bridges, sclerotia through rain splash, and infected soil by infected plant debris and basidiospores as air borne (Sian and Kumar, 2018).
Utilization of different methods to control web blight are required, this is because the variability of this disease to infect range of host. Furthermore, used of fungicide that may lead to environment and soil pollution. Many studies showed that there is an effect of sulphate in reducing the incidence and disease severity (Saeed, 2016). Hiruma et al; 2013, mentioned that the hyper sensitive protein increased by increasing the glutathione rate and that due to increasing the sulphate amount. However, Trichoderma harzanium as well showed the ability of it in reducing the the severity of many disease such as Fusarium wilt in banana crop (Castillo et al; 2019), pathogenicity of Botrytis cinerea (Zimand et al; 1996), and Benhamou and Chet, 1993, found that the growth of pathogen inhibit by interacting with Trichoderma harzianum. As well as, Psedomonus fluorescens showed

an effect on some disease such as soil borne disease and a plant growth promoting (Girija and Kumar , 2005; and David et al; 2018).

Therefore, to management in eco-friendly way and use some biological agent of web blight are required. Thus, present investigation was aimed to study the cultural, morphological and pathological variability of utilization different method to control R. solani causing web blight of mung bean.

Materials and methods

Isolation of R. solani and culturing

Isolated of R. solani was done by taking a part of leaf that infected by it, sterilized with hypo chloride sodium (3%) then rinse with DDW (Deionized distilled water) three time then incubated for 48 hours in a potato dextrose agar, after the conidia appear, moved to new culture of PDA, stored until use. Microscopy test was done to identified that is R. solani.

Biological agent and fertilizer

Two different biological agents were used in these two experiments (Trichoderma harazanium and Pseudomonas fluorescens) and potassium sulpahte as a fertilizer treatment, this used 60 kg/ha as S. PDA and NA used to re culture the both T. harazanium and P. fluorescens respectively. R solani culture was inoculated the soil and seedling into two different ways to make an infection. One by infected the seed of panicum miliaceum then applied to the soil at 3-5 cm depth, and the second was by spring the spore suspension to seedling.

In vitro:

Multi formulation were used to test the ability of treatment to inhibition of disease. Three replicates of each formulation were done, through make each formulation, for sulpahte 0.6 mol was dissolved then add to the PDA culture and 5 cm dimeter of culture of R. solani put in the middle of plate, for Bio 1 after preparing the culture of PDA Bio was add to this then a 5cm dimeter of R. solani put it in the middle of the plate,this id repeate for Bio 2 as well. This way repeated for test the sulphate with Bio 1 and Bio 2 to investigate if there is any effect of sulphate with Bio 1 or Bio 2.

Field experiment

Field experiment was conducted to investigated these treatments under field condition, with randomized completely block design, this include two different cultivars and 8 treatments for each variety (local and Brazilian) (Table 1).

Table (1). the treatments and its abbreviation that used in field experiment.

Treatment	Abbreviation
P. *fluorescens*	Bio1
T. harzanium	Bio 2
Fertilizer	F
Control -	Without all
Control +	Ch
P. fluorescens* and T. *harzanium	Bio 1 + Bio 2
***P. fluorescens* + fertilizer**	Bio 1 + F
***T. harzanium* + fertilizer**	Bio + F

After sowing the inoculation was added at 5cm depth near the seedling root (rhizosphere), in fortnight application of treatments were applied. Fortnight later some measurements of disease, vegetative and yield. Disease measurement include disease incidence and severity, these through take the number of infection plant and number of all plant at 1square meter, while severity through make a disease index (Table 2).

Results:

In vitro

In vitro result showed that there is a high reduction in diameter of fungus mycelium which reduced by 86.6% and 88.6% in both T. harzianum and P. fluorescens respectively, table (3).

Table (3) percentage and diameter of inhabitation of R. solani in two different organisms in vitro

Treatment	Inhabitation /cm	% of inhibitation
T. harzanium	2	86.6
P. fluorescens	1.7	88.6

Disease incidence and severity

All treatments reduced disease incidence in comparison with negative control. Although significant differences achieved with all treatments, Bio2 reduced two disease incidences by 96.8% and 94.6% respectively. In addition, disease severity showed that there are not statistically differences between treatments in comparison with negative control. However, there is differences between these treatments. (Table 4).

Table (4) disease incidence in two different time after application of treatments and disease severity for field experiment.

Treatment	Incidence 1	Incidence 2	Disease severity
with bio1	30.6	4.3	0.26
with bio1 with bio2	35.7	4.6	0.81
with bio2	26.9	2.4	0.45
with che	28.1	4.3	0.74
with Disease only without any treatments	89	44.8	1.1
with f1	31.4	4	0.39
with f1 with bio1	33.8	3.9	0.71
with f1 with bio2	29.2	3.3	0.51
L.S.D.	10.77	3.33	0.7

Chlorophyll rate

Result showed that there is a significant difference between them, which Bio 2 increase the rate of chlorophyll to reach 35.52 and this rate same with positive control (chemical treatment). In addition, other treatments (with F, with F and Bio 1, and with F and Bio2) which not differences with positive control as well. While Bio1 and Bio1 with Bio2 achieved 30.5 and 29.2 respectively, and that be less rate in comparison with other treatments (Figure 1).

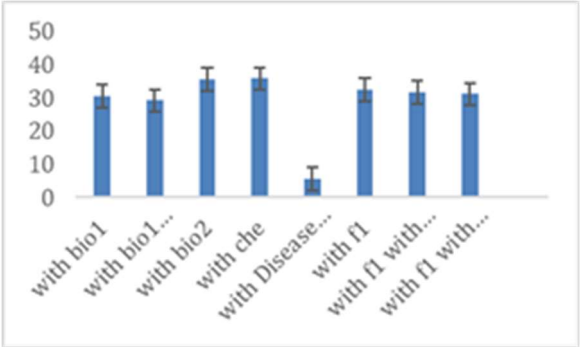

Figure (1). Rate of chlorophyll for field experiment that treated with different treatments (P. <0.001, L.S.D. 3.4).

wet and dry weight of root and vegetative

Variation of wet and dry weight of each of root and plant vegetative showed that there is a significant difference between the treatments which utilization with this experiment. Dry and wet weight were differed in comparison with negative control which increased by 21.6 % and 22.83% of F with Bio2 treatment respectively. Furthermore, F treatment alone achieved high dry weight of root and plant vegetative which increased by 10% and 17.82% respectively. (Table 5).

Table (5). Effect of treatments in dry and wet weight of each of root and vegetative in field experiment.

variation/ treatments	dry weight root	dry weight vegetative	root wet weight	vegetative wet weight
with bio1	12.23	22.72	26.48	39.1
with bio1 with bio2	14.13	26.42	31.5	46.7
with bio2	14.05	25.97	33.65	48.7
with che	14.03	23.5	26.45	36.85
with Disease only without any treatments	1.78	5.02	7.58	11.45
with f1	17.65	28.17	30.95	43.1
with f1 with bio1	15.45	26.42	31.3	41.2
with f1 with bio2	15.9	27.65	35.08	50.15
L.S.D.	3.016	3.067	8.284	1.71
P value	<0.001			

Dry weight of root and vegetative

Both cultivars showed that significantly difference of the wet and dry weight of each of root and plant vegetative, in all variations local cv. gave a high weight of dry weight of root and plant vegetative and wet weight of both 14.68, 25.94, 30.49, and 45.72 gm respectively. figures (2,3,4, and 5).

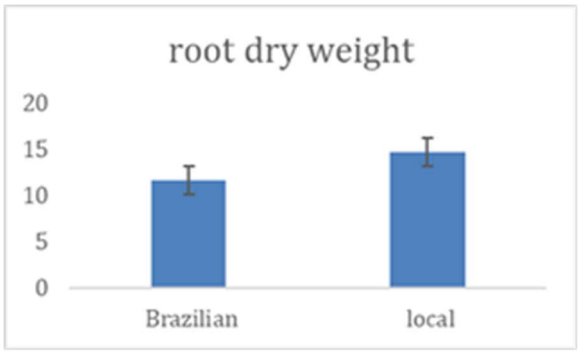

Figure (2). Differences between cultivars (Local and Brazelian) in dry weight of root. (L.S.D. 1.50, P value <0.001).

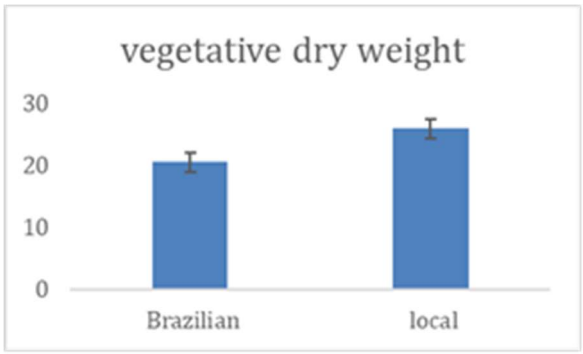

Figure (3). Differences between cultivars (Local and Brazelian) in dry weight of plant vegetative. (L.S.D. 1.53, P value <0.001).

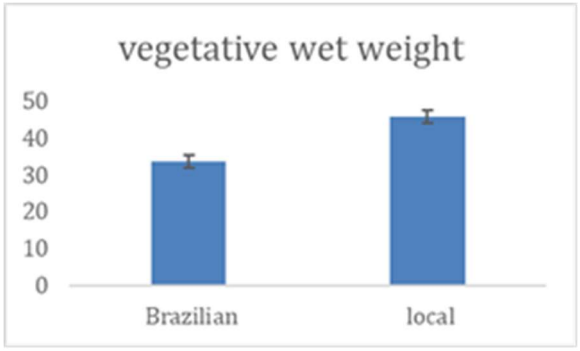

Figure (4). Differences between cultivars (Local and Brazelian) in wet weight of plant vegetative. (L.S.D. 1.82, P value <0.001).

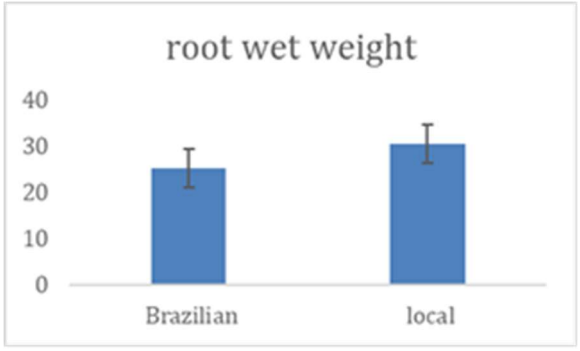

Figure (5). Differences between cultivars (Local and Brazelian) in wet weight of plant vegetative. (L.S.D. 4.14, P value <0.017).

Interaction between treatments and cultivar on vegetative wet weight

Local cv. showed that there is a statically differences in comparison with Brazilian cv. with all treatments. In addition, F with Bio2 treatments achieved a high value with local cv. 61.6 gm. Also, Bio2 alone and Bio2 with Bio1 treatments increased the wet weight by 79.5% and 80% respectively.

Table (6). Effect of the interaction between the treatments and cultivars on vegetative wet weight in field experiment.

Treatments /cv.	Brazilian	local
with bio1	33.35	44.85
with bio1 with bio2	35.8	57.6
with bio2	41.25	56.15
with che.	35	38.7
with Disease only without any treatments	11.4	11.5
with F	37.6	48.6
with F with bio1	35.65	46.75
with F with bio2	38.7	61.6
L.S.D.	2.418	
P. value	<0.001	

Effect of treatments on no. and weight of pods and yield.

Significant differences achieved with all treatments on number of pods, pods weight, plant high, and total yield. Formulation of F with Bio2 treatment increased each of all variation in this study (Table 7), which increased by 65.4%, 86.5%, 49.5%, and 74% respectively in comparison with negative control. In spite of there are differences between other variations, there is not significantly differences between them. But these significantly differences in comparison with negative control.

Table (7). Effect of treatments on each of number of pods, pods weight, plant high, and total yield per unit at field experiment.

treatments/ variations	no of pods	plant high	pods weight	total yield of second harvest
with bio1	80	57.4	24.52	27.2
with bio1 with bio2	85.5	35.2	23.73	24.1
with bio2	84.75	76.6	23.88	24.4
with che	84.25	49.3	24.9	27
with Disease only without any treatments	31.75	13	14.35	8.6
with f1	78.75	63.4	23.62	30.5
with f1 with bio1	84.75	85.9	26	30.8
with f1 with bio2	91.75	96.7	28.4	33.1

lsd	6.393	23.08	4.576	9.3
P. value	<0.001	<0.001	<0.001	<0.001

Discussion:

In vitro experiment

Both biological treatments make an inhabitation in a dimeter of fungus disease this is might be due to the competition of these. P. fluorescens known as plant growth promoting regulator (PGPR) and plant growth has improved by that in different mechanisms, this included siderophores production, produce plant growth hormone, enhance plant uptake of mineral, and synthesis of antibiotics (Glich 1995). This bioagent also being as a potential crop promoting against fungus disease (Zegeye et al. 2011). In addition, T. harzianum may inhabit the rhizosphere, taking advantage of root exudates; others may live on root or leaf surfaces and some may colonize intracellular spaces and vascular tissues inside the plant (Preston, 2004).

Field experiment:

Field results showed that the Bio1, Bio 2, fertilizer have decreased disease incidence. However, other variations dramatically increased by these treatments. That might due to the effect of the biocontrol agent that they know as T. harzianum and found an effective plant disease management (Harman, 2000). In addition, the role of P. fluorescens as PGPR, the study done by Borowicz et al (1992), mentioned the ability of this biological agent in inactivate of cell wall degrading enzymes of plant against fungus. Another study by Hebbar et al. (1992), proved that antifungal activity of P. fluorescens in producing antibiotic which is mainly responsible about it. Furthermore, P. fluorescens produced 2,4-diacetyl phloroglucinol as anti-fungal metabolite which is play an important role in biological control against plant pathogens (Delany et al. 2000).

Fertilizer have been known as enhancing plant growth as a nutrient supply. Tao et al (2020) found that plant resistance improved by organic fertilizer. The use of plant probiotic microorganisms has been shown to hold promise for improving plant health, nutrition, and stress resilience (Berendsen et al. 2012, Berlec 2012, Wei et al. 2015), and the delivery of such plant probiotics via for instance bioorganic fertilizers has proven particularly effective in improving soil microbial functionality (Marcano et al. 2016, Xiong et al. 2018). In spite of potentially effective, the mechanisms driving the success of such bioorganic fertilizer applications are generally not well described. Many modes of action are explaining the possibility of the role of that, including direct antagonism of the pathogen (Harman et al. 2004, Haas and Défago 2005), induction of systemic resistance in plants (ISR) (Haas and Défago 2005, Weller 2007) or indirect impacts on the pathogen via effects on the resident soil microbiome (Xiong et al. 2017, Fu et al. 2017). In addition, Sulphur fertilizer has been showed evidence that there is an effect on some plant disease for instance Zymoseptoria tritici and Parastagnospora nodorum, and Alternaria alternata (Saeed, 2016, Al-jubouri and Saeed, 2020). However, the possibility of how its effect to against disease unclear. Thus, more investigation is required to explain the mechanism of that.

References:

Glick BR (1995) Enhancement of plant growth by free living bacteria. Can J Microbiol 41:109–117.

Berendsen RL, Pieterse CM, Bakker PA (2012). The rhizosphere microbiome and plant health. Trends Plant Sci. 17:478–86.

Berlec A. Novel techniques and findings in the study of plant microbiota (2012): search for plant probiotics. Plant Sci. 193-194:96–102.

Wei Z, Yang T, Friman VP, Xu Y, Shen Q, Jousset A. (2015). Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. Nat Commun. 6:8413.

Marcano I-E, Díaz-Alcántara C-A, Urbano B, González-Andrés F. (2016). Assessment of bacterial populations associated with banana tree roots and development of successful plant probiotics for banana crop. Soil Biol Biochem. 99:1–20.

Xiong W, Jousset A, Guo S, Karlsson I, Zhao Q, Wu H, et al. (2018). Soil protist communities form a dynamic hub in the soil microbiome. ISME J. 12:634–8.

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. (2004). Trichoderma species--opportunistic, avirulent plant symbionts. Nat Rev Microbiol. 2:43–56.

Haas D, Défago G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol. 3:307–19.

Weller DM. (2007). Pseudomonas biocontrol agents of soilborne pathogens looking back over 30 years. Phytopathology. 97:250–6.

Xiong W, Guo S, Jousset A, Zhao Q, Wu H, Li R, et al. (2017). Bio-fertilizer application induces soil suppressiveness against fusarium wilt disease by reshaping the soil microbiome. Soil Biol Biochem. 114:238–47.

Fu L, Ruan Y, Tao C, Li R, Shen Q. (2016). Continous application of bioorganic fertilizer induced resilient culturable bacteria community associated with banana fusarium wilt suppression. Sci Rep. 6:27731.

Chengyuan Tao, Rong Li1, Wu Xiong, Zongzhuan Shen1, Shanshan Liu1, Beibei Wang, Yunze Ruan, Stefan Geisen, Qirong Shen1, and George A. Kowalchuk (2020). Bio-organic fertilizers stimulate indigenous soil Pseudomonas populations to enhance plant disease suppression. Microbiom 8:137.

Borowicz JJ, Pietr SJ, Stankieka Z (1992). Inhibition of fungal cellulase, pectinase and xylanase activity by plant growth-promoting fluoresces Pseudomonas spp. Bulletin OILB SROP 15:103-106.

Delany I, Sheehan MM, Fenton A, Bardin S, Aarons SO, Gara F (2000). Regulation of production of the antifungal metabolite 2,4- di acetyl phloroglucinol in Pseudomonas fluorescens F113: genetic analysis of ph1f as a transcriptional repressor. Microbiol reading 146: 537546.

Hebbar KP, Davey AG, Dart PJ (1992). Rhisobacteria of maize antagonistic to Fusariun moniliforme pathogen; isolation and identification. Soil Biol Biochem 24:979987.

Preston GM (2004). Plant perceptions of plant growth-promoting Pseudomonas. Trans. l Soc. London B.359: 907–918.

Zghair QN, Lal A, Nane MM, Sobita S (2014) Effect of bioagents and fungicide against early blight disease of tomato (Lycopersicon esculentum L.). international. Tunis J Plant Prot 7:330 – 333.

Harman GE (2000). Myths and dogmas of biocontrol: changes in perceptions derived from research on Trichoderma harzianum T-22. Plant Dis 84: 377-393.

Arcia, R.N., Adachi, M., Maruyama, Y., Tecson-Mendoza, E.M., Mikami, B., et al., (2006). Structure of 8Sα globulin, the major seed storage protein of mung bean. Acta Crystallogr. D. Biol. Crystallogr. 62: 824–832.

Singh, J., Singh, R.B. and Balai, L.P. (2012). Grain Yield Loss in Mungbean due to Web Blight. Trends in Biosciences, 2:147-148.

Gupta, R.P., Singh, S.K. and Singh R.V. (2010). Assessment of losses due to web blight and weather effects on disease development in mung bean. Indian Phytopath., 1:108-109.

Saeed, Khaldoon Faris SAEED, (2016). Effect of Sulphur and silicone fertilizer on disease control and quality and yield of wheat. PhD thesis. Reading university, Reading, UK.

Hiruma, K., Fukunaga, S., Bednarek, P., Mariola, P., Watanabe,S., Narusaka, Y., Shirasu,K., and Takano, Y. (2013). Glutathione and tryptophan metabolism are required for Arabidopsisi immunity during the hypersensitive response to hemibiotrophs. PANS 110: 9589-9594.

Benhamou, Nicole and IIan Chet. (1993). Hyphal interactions between Trichoderma harzianum and Rhizoctonia solani: Ultrastructure and Gold cytochemistry of the Mycoparasitic process. Phytopathology 83: 1062-1071.

Arfe G. Castillo, Cecirly G. Puig and Christian Joseph R. Cumagun. (2019). Non-Synergistic Effect of Trichoderma harzianum and Glomus spp. in Reducing Infection of Fusarium Wilt in Banana. Pathogens 8: 43.

Gilly, Zimand; Yigal, Elad; and IIan Chet. (1996). Effect of Trichoderma harzianum on Botrytis cinerea Pathogenicity. Phytopathology 86: 1255-1260.

Girija Ganeshan and A. Manoj Kumar. (2005). Psedomonus fluorescens, a potential bacterial antagonist to control plant disease. Journal of plant Interactions 3:123-134.

Baliah V, David, Govindan Candrasehar, Pamila N. Selvam. (2018). Chapter 10- Pseudomonas Fluorescens: A Plant-Growth-Promoting Rhizobacterium (PGPR) with potential role in biocontrol of pests of crops. Crop improvement through Microbial Biotechnology https://doi.org/10.1016/B978-0-444-63987-5.00010-4.