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EFFECT OF PGPM IN CONTROLLING SEED GALL DISEASE CAUSED BY *ANGUINA TRITICI* ON WHEAT PLANTS

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Abstract

A pot experiment was conducted during 22 November 2018 at plant protection directorate, Abu-Ghraib-Iraq to Evaluate the efficiency of some microorganisms (*Pseudomonas fluorescence*, *Bacillus subtilis*, *Trichoderma harzianum*, *Verticillium* sp., *Paecilomyces lilacinus*) used singly or in combination to control seed gall disease. Wheat seed IPA-99 variety was planted in pots filled with sterilized soil, while galls were buried between seeds. Before 48h from sowing (100ml.pot^{-1}) of microbial culture was add to each pot. The experiment was designed in a complete random design CRD with four replicates, growth parameters was taken at flowering stage while yield parameters taken at harvest (8 May 2019). Results indicate reduce disease incidence of seed gall disease on wheat plants, it ranged from 0.000% to 8.020% with a significant differences from control treatment, Moreover results showed the effect of PGPM for increase flag leaf area and plant height of all treatments with a significant difference from control. Although maximum value of grain yield was 16.80g/pot, but the highest value of spike weight was ranged between 2.443g -2.133g and the highest value of biological yield ranged from 85.33g to 79.00g.

Key words: PGBM, Seed gall disease, *Trichoderma harzianum*, *Paecilomyces lilacinus*

Introduction

Wheat *Triticum aestivum* L. is one of the strategic globally crops constitutes the key sources of protein in least developed countries and middle-income nations. It is one of the principle cereal crops which was grown worldwide and one of the important staples of nearly 2.5 billion of world population. Wheat is the major staple food crop that providing almost half of all calories in North Africa as well as west and central Asia, being next to rice. Globally wheat occupies around 217 million hectares so it is holding the position of highest acreage among all crops and recorded annual production hovering around 731 million tones (USDA, 2018), in Iraq at 2019 wheat cultivated area reaches 6331000 donnums, with a total production 4343 tones (CSO, 2019), Iraq currently consumes nearly 4 million tons of wheat annually (USAID, 2006). Moreover wheat like any other plant infected by many diseases, seed gall was one of the oldest reported diseases on wheat caused by *Anguina tritici* (Bhatti *et al*., 1978), sometime it named seed-gall, Gout, Purples, False (Esser *et al*., 1991). Unfortunately, seed gall disease could reduce human consumption and market price of wheat due to the reduction of protein and gluten content of flour produced from wheat infected with seed galls (Mustafa, 2009). Scientists indicate several practices for reduce nematodes infection such as physical, mechanical, chemical and agricultural methods (ISPM, 2017) which is considered traditional controlling ways, but recently biological methods considered the best management in which microorganisms regarded as an alternative proposal for agriculture sustainable and development, it is the better, cheaper and more environmental friendly alternative processes for controlling the specific agricultural problems and increase food productions (Hayat *et al*., 2010). Different authors have reviewed the properties and traits of some plant growth promoting rhizospheric microorganisms (PGPM) by their effect on promote the increase of nutrient supply and used as bio-fertilizers in addition to bio-pesticide properties (Tabassum, 2017). PGPM consist of a large group of microorganisms that can be found in the rhizosphere nearby the root surface or associated to it (Basu *et al*., 2017; Gupta *et al*., 2018). Bacteria and fungi are fundamental and essential part of soil ecosystem and their presence is beneficial for plant growth to keep soil environment rich in all kinds of macro and micro nutrients which lead at the end to increase plants fitness (Chun-Li *et al.*, 2014). In recent years it used as bio-control against pathogenic organisms due to their antagonistic properties to plant pathogens (Chauhan *et al.*, 2015). Sikora (1992) suggested the term antagonistic potential for all parasites, predators, pathogens, competitors and other organisms in soil that works together to repel and inhibit or even kill plant parasitic nematodes. Antagonists most likely to be receptive to management for the biological control of nematodes are: predacious or trapping fungi; endo-parasitic fungi; fungal-pathogen/parasites of females, endo-mycorrhizal and mutualistic fungi; plant-health promoting rhizobacteria and obligate bacterial parasites (Beneduzi *et al.*, 2012). Actually, the antagonistic activities of bio-control agent can effectively suppress, reduce or eliminate nematode diseases incidence through a number of ways such as Production of hydrolytic enzymes:

- Competition for nutrients.
- Production of antibiotics.
- Modulate ethylene levels caused by pathogenic infection.

(Carlos M. H. Ferreria *et al.,* 2019).

The aim of this study was to Evaluate the efficiency of (*Pseudomonas fluorescence*, *Bacillus subtilis*, *Trichoderma harzianum*, *Verticillium* sp., *Paecilomyces lilacinus*) used singly or in combination to control seed gall disease caused by *Anguina tritici* on wheat plants.

Materials and Methods

A pot experiment was conducted during 22 November 2018 at plant protection directorate, Abu-Ghraib. Wheat seed IPA-99 variety (obtained from agriculture research directorate, ministry of agriculture) was planted (10 seed/pot) in pots which was filled with 15Kg sterilized loamy soil (1% formalin 1L.m^3 soil). Galls were buried between seeds at the rate of one gall/hole; all treatments were inoculated with 30 wheat galls/pot (Yonis, 2015) except T33, T34. The concentration of bacterial suspension *P. flourescens* and *B. subtilis* was adjusted to 1×10^8cfu.ml^{-1}, while *T. harizianum*, *Verticillium* sp. and *P. lilacinus* 1×10^7spor.ml^{-1}. Microorganisms was add (100ml.pot^{-1}) before 48h from sowing. The experiment was designed in a complete random design CRD with four replicates (pots) for each treatment, growth parameters was taken at flowering stage after 120 day from sowing while yield parameters taken at harvest (8 May 2019).

Treatments

T1: *Pseudomonas flourescens* (P.f.)+30 gall.pot^{-1}.
T2: *Bacillus subtilis* (B.s.) +30 gall.pot^{-1}.
T3: *Trichoderma harizianum* (T.h.) +30 gall.pot^{-1}.
T4: *Verticillium* sp. (V. sp.) +30 gall.pot^{-1}.
T5: *Paecilomyces lilacinus* (P. l.) +30 gall.pot^{-1}.
T6: P.f. + B.s +30 gall.pot^{-1}.
T7: P.f. + T.h. +30 gall.pot^{-1}.
T8: P.f. + V. sp. +30 gall.pot^{-1}.
T9: P.f. + P. l. +30 gall.pot^{-1}.
T10: B.s. + T.h. +30 gall.pot^{-1}.
T11: B.s. + V. sp. +30 gall.pot^{-1}.
T12: B.s. + P. l. +30 gall.pot^{-1}.
T13: T.h. + V. sp. +30 gall.pot^{-1}.
T14: T.h. + P. l. + 30 gall.pot^{-1}.
T15: V. sp. + P. l. + 30 gall.pot^{-1}.
T16: P.f. + B.s. + T. h. +30 gall.pot^{-1}.
T17: P.f. + B.s. +V. sp. +30 gall.pot^{-1}.
T18: P.f. + B.s. + P. l. +30 gall.pot^{-1}.
T19: B.s. + T.h. + V. sp. +30 gall.pot^{-1}.
T20: B.s. + T.h. + P. l. +30 gall.pot^{-1}.
T21: T.h. + V. sp. + P. l. +30 gall.pot^{-1}.
T22: P.f + T.h. + V. sp. +30 gall.pot^{-1}.
T23: P.f. + V. sp. + P. l. +30 gall.pot^{-1}.
T24: B.s. + V. sp. + P.l. +30 gall.pot^{-1}.
T25: P.f. + T.h. + P. l. +30 gall.pot^{-1}.
T26: P.f. + B.s. + T.h. + V. sp. +30 gall.pot^{-1}.
T27: P.f. + B.s. + T.h. + P. l. +30 gall.pot^{-1}.
T28: B.s. + T.h.+ V. sp.+ P. l. +30 gall.pot^{-1}.
T29: P.f. + T.h.+ V. sp.+ P. l. +30 gall.pot^{-1}.
T30: P.f. + B.s. + V. sp. + P. l. +30 gall.pot^{-1}.
T31: P.f. + B.s. + T.h. + V. sp. + P. l. +30 gall.pot^{-1}.
T32: 30 gall.pot^{-1} + 10 wheat seed. (Control)
T33: 10 wheat seed only. (Control)
T34: P.f.+ B.s. + T.h.+ V. sp.+ P. l. + 10 wheat seed. (Control)

Agronomic traits

Flag Leaf area (cm^2)

Flag leaf area of 10 random plants from each treatment was measured by the following equation (Thomas, 1975).

Leaf area (cm^2) = leaf length (cm) × leaf width (cm) × 0.95.

Plant height (cm)

Plant height was measured by recorded the average of 10 random plants from each treatments was measured from plant base to the tip of main stem spike excluding awns (Singh and Stoskopf , 1971).

Yield and yield components

When the crop plants matured, spikes from each pot were collected to determined average spikes weight per pot and then the spikes were threshed to count (grain weight, grains number, galls number) per pot.

Biological yield

Biological yield was calculated by summation of straw and grain weight per pot.

Disease incidence of seeds (%)

Disease incidence was measured according to the following equation:

Disease incidence % = number of galls ÷ (number of galls + number of grain) × 100

Statistical analysis

Statistical analysis was carried out by using Genstat computer program with 0.05 significance level (95% confidence).

Results

Effect of PGPM to control seed gall disease on wheat plants

Results indicate the efficiency of PGPM in reduce disease incidence of seed gall disease caused by *A. tritici* on wheat plants with a significant differences from control treatment table 1. Disease incidence ratio ranging from 0.000% to 8.020%. T-15, T-16, T-17, T-18, T-21 recorded 0% disease incidence with a significant differences from other treatments fallowed by T-19 which recorded 0.08% disease incidence with a significant differences from other treatments, while disease incidence ranging from 0.13% to 8.02% for other treatments with a significant differences from control treatment (T-32 free of microorganisms).

Effect of PGPM on some wheat agronomic traits that infected by seed gall nematodes

Results showed the efficiency of PGPM for increase flag leaf area of all treatments with a significant differences from control treatment (T32), while T17, T18 exceed significantly than other treatments without significant differences between them 38.50, 38.60 respectively, followed by T10 and T11which recorded 34.83, 35.28 respectively, moreover the minimum value of flag leaf area was 19.36 recorded in control treatment (T32). Results revealed the effect of PGPM on wheat infected plant height, however all treatments was a exceeded with a significant differences than control treatment, but the highest value of plant height 89.00cm was recorded in T6 with a significant differences than other treatments, followed by T5 and T18 which recorded 85.00cm, 84.75cm respectively without significant differences between them, in addition the lowest value was 42.75cm recorded in control treatment (T32) table 1.

Table 1: Effect of PGPM on control seed gall nematode disease and some agronomic traits, yield of wheat plants infected by seed gall nematodes.

Treat.	Disease incid-ence (%)	Flag leaf area (cm^2)	Plant height (cm)	Grain yield (gm/ pot)	Biolo-gical yield (g/pot)	Spike weight (g)
T -1	4.327	26.40	75.00	6.26	57.67	1.302
T-2	1.407	23.44	75.25	6.70	70.67	1.540
T-3	0.453	27.50	76.50	7.03	60.5	1.573
T-4	0.183	31.06	79.50	12.4	79.00	2.093
T-5	8.020	25.79	85.00	13.86	83.83	2.437
T-6	2.553	27.39	89.00	9.10	71.67	1.680
T-7	0.130	25.34	83.00	14.43	81.67	1.896
T-8	1.310	28.02	83.75	13.03	80.00	2.383
T-9	0.210	30.59	80.50	9.20	68.50	1.970
T-10	0.650	34.83	73.75	15.36	85.33	2.443
T-11	3.487	35.28	72.25	7.90	52.33	1.877
T-12	0.683	31.30	64.75	12.60	73.67	2.167
T-13	1.487	29.63	63.75	14.93	81.00	2.230
T-14	0.210	23.52	65.75	10.63	59.83	1.223
T-15	0.000	26.46	62.75	13.23	68.33	2.017
T-16	0.000	26.70	62.75	12.60	71.00	2.133
T-17	0.000	38.50	82.00	13.03	69.17	1.644
T-18	0.000	38.60	84.75	13.23	64.67	1.467
T-19	0.080	29.53	79.75	13.57	69.67	1.713
T-20	0.193	25.68	72.75	9.00	55.83	1.633
T -21	0.000	21.20	63.00	13.50	68.00	1.560
T-22	0.180	25.47	62.00	11.70	60.33	2.133
T-23	0.597	30.54	72.50	11.77	59.33	1.903
T-24	1.523	32.49	74.75	8.10	46.00	1.393
T-25	0.763	25.75	68.25	11.33	63.17	1.223
T-26	0.473	28.83	63.50	12.79	62.20	1.790
T-27	0.240	22.34	58.25	11.30	60.83	1.227
T-28	0.693	31.47	78.50	13.77	61.83	1.087
T-29	0.967	21.88	61.25	13.86	66.33	1.160
T-30	1.220	30.84	74.75	8.57	46.67	1.333
T -31	0.600	24.57	76.25	16.80	77.33	2.177
T-32	18.867	19.36	42.75	5.10	45.83	0.740
T-33	0.000	38.94	93.75	20.40	80.00	2.583
T-34	0.000	45.66	94.00	21.63	94.00	2.687
L.S.D	0.0429	3.054	3.454	2.025	8.469	0.3065

Effect of PGPM on yield of wheat plant infected by seed gall nematodes

The effect of PGPM on grain yield recorded exceed all treatments significantly than control treatment (T32), Maximum value was 16.80 g/pot recorded in T31 without significant differences than T10, T13 which recorded (15.36, 14.93) g/pot respectively, while there is no significant differences in grain yield between T13 and T7, T5, T8, T15 T17, T18, T19, T21, T28, T29 which recorded (14.43, 13.86, 13.03, 13.23, 13.03, 13.23, 13.57, 13.50, 13.77, 13.86) g/pot respectively, moreover the minimum value was 5.10g/pot recorded by control treatment (T32). Results also showed the efficiency of PGPM on spike weight for all treatments with a significant differences from control treatment (T32), The highest value was (2.443, 2.437, 2.383, 2.167, 2.230, 2.177)g recorded in T10, T5, T8, T12, T13, T31 respectively without significant differences between them (except T33 and T34), followed by T22, T16, T7, T9, T11, T23 which recorded (2.133, 2.133, 1.896, 1.970, 1.877, 1.903)g respectively, and minimum value was 0.740g recorded in control treatment (T32). Moreover results revealed the effect of PGPM on biological yield of wheat plants, however all treatments was exceeded with a significant differences than control treatment, but the highest value of biological yield 85.33, 79.00, 83.83, 81.67, 80.00, 81.00, 77.33 was recorded in T10, T4, T5, T7, T8, T13, T31 respectively without significant differences between them, followed by T2, T6, T12, T16, T17, T19 which recorded 70.67, 71.67, 73.67, 71.00, 69.17, 69.67 respectively without significant differences between them, in addition the lowest value was 45.83gm/pot recorded in control treatment (T32) table 1.

Discussion

These results agreed with the findings of many researchers, AL-Taie, (2018) revealed that bio-pesticide has the ability to decrease the infection percentage 77.78%, while Shinya *et al*., (2008) observed the positive effect of *Verticillium lecanii* to reduce nematode (*Heterodera glycines*) eggs density by 93.2%, while Hussain *et al*., (2018) confirm the benefit of *Lecanicillium muscarium* a hybrid of *Verticillium* sp. on controlling eggs and J2 of *Meloidogyne incognita*, on the same subject Sharon *et al*., (2001) results agreed on use of *Trichoderma harzianum* as biological control against *Meloidogyne javanica* due to its mortal effect on eggs and J2, while results of Samaraj *et al.,* (2014) confirmed on the use of *Pseudomonas fluorescens* to control root-knot nematodes in chillies. Moreover, Several researchers concluded the positive combined effect of microorganisms for controlling nematodes, Anastasiadis *et al*., (2008) observed the effect of *P. lilacinus* and *Bacillus firmus* on suppression J2 nematodes which recorded 58% and 66% after 14 day respectively. Furthermore these results agreement with those obtained by Mohammed *et al.,* (2012) who recorded significant increase in wheat growth and yield by 36% due to the presence of *Azospirillium brasilense* and *Bacillus polymyxa* as bio-fertilizers, These results also agreement with those obtained by Ozberk *et al*., (2011) who reported that average spike weight in healthy wheat plant recorded 2.79g while it reach 0.55g in infected plant by *A. tritici*, furthermore Mohamedova and Piperkova (2013) reported that there was a significant decrease in yield between a healthy wheat plant and infected wheat with seed gall disease caused by nematode *A. tritici*, it also agreed with Singh *et al*., (2019) results who reported a significant decrease in disease incidence 17.46% caused by *Bipolaris sorokinina* on bread wheat and increase in yield when seeds inoculated by *Trichoderma harzianum*. Moreover, Rao *et al*., (2017) results recorded maximum increase in carrot yield 28.8% and decrease in root-knot nematode population 69.3% by using vermicompost enriched with *Bacillus subtilis*.

Controlling nematodes by PGPM could be in direct or indirect methods, the direct one depending on the bacterial and fungal positive reductional effect on J2 by physical application throw capturing them by fungal trapping network, or chemical application due to the production of secondary metabolites such as antibiotics, HCN, volatile compounds (Samarags and Hair, 2014) in addition to bacitracin, subteolin, benzene acetaldehyde (Killani *et al*., 2011), chitenase, protease, glucanase (Nagar and Anand, 2014), whilst the indirect methods was throw induced systemic resistance by production of several compounds like lipo-polysaccharides, N-alkylated benzoamin derivatives, 2, 3-butanidol, pyocyanin which aid in the structural modification occurring in plant cell wall as well as triggering for synthesis of phytoalaexins, sesquiterpenoids and isoflavinoid (Corné *et al.,* 2014).

PGPM enhancing plant growth due to the availability of different nutrients including N, P and K in addition to several micronutrients, synthesize siderophores that sequester iron from the soil and provide it to plants (Sah and Singh, 2015), synthesize several different phytohormones to enhance various stages of plant growth such as IAA, gibberellin and cytokinin (Al-Taie, 2018; Carlos, 2019), some fungi filaments improving plant growth by secretion growth hormones beside some other mechanisms in addition to produces growth factors that enhanced seed germination, plant growth and yield.

Furthermore, increase the impact of nitrogen, phosphorus and potassium in dry matter of plants tissue. increasing available P in soil by production of organic acids and phosphatase enzymes, it was also able to release low molecular weight organic acids mainly gluconic and keto gluconic acids which through their hydroxyl and carboxyl groups chelate the cations (Al, Fe, Ca) bound to phosphate, moreover it could be increasing Fe solubility and hence uptake by plant, all these function lead to increase fitness of the plant and improve its growth (Gupta *et al*., 2018) which lead finally to increase plant vigor for facing pathogens.

Conclusion

Bio-control management considered the best in which microorganisms regarded as an alternative proposal for agriculture sustainable and development, it is the better, cheaper and more environmental friendly alternative processes for controlling specific agricultural problems and increase food productions The present study proves the successful combination of functional microorganisms for effective management of seed gall disease, it also proves the antagonistic potential of *Pseudomonas fluorescence*, *Bacillus subtilis*, *Trichoderma harzianum*, *Verticillium* sp., *Paecilomyces lilacinus* against *Anguina tritici* nematode in wheat plant.

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