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ORIGINAL PAPER

Potential of Biotic and Abiotic Inducers of Eliciting Systemic Resistance in Snap Bean (*Phaseolus vulgaris* L.) in Controlling Rust Caused by *Uromyces appendiculatus*

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ABSTRACT

The biotic and abiotic inducers of resistance *i.e.*, *Bacillus subtilis*, *Paenibacillus polymyxa*, *Trichoderma harzianum*, *Trichoderma viride*, ascorbic acid, Bion, potassium citrate, salicylic acid, as well the fungicides *i.e.*, Domark EC 10% and Tilt EC 25%, were evaluated *in vitro*, and field conditions in both 2019 and 2020 growing seasons to control snap bean rust (*Uromyces appendiculatus*) at Ashmoun, Menoufia Governorate, Egypt. *In vitro* results indicated that all the tested bio-agents, chemical resistance inducers and the two fungicides significantly inhibited uredospores germination. The fungicide Domark EC 10% was the best effective treatment followed by Tilt EC 25% fungicide and *B. subtilis*, where they recorded 97.1, 96.3 and 82.4% inhibition of uredospore germination percentage, respectively, comparing with control treatment. Spraying the tested biotic, abiotic inducers, and the tested fungicides of bean plants under field conditions significantly reduced disease severity of bean rust, Area Under Disease Progress Curve (AUDPC) and rate of disease increase (r-value). Meanwhile, significantly increased performances of growth and yield parameters *i.e.*, plant height, No. of branches/plant, plant fresh and dry weights, pod length (cm), average pod weight/plant (g) and yield (ton/fed), in both 2019 and 2020 growing seasons compared to the untreated control treatment. Meantime, considerable increases were recorded resulted in all the tested treatments in photosynthetic pigments, chlorophyll and carotenoids; activities of peroxidase (PO) polyphenol oxidase (PPO) and Catalase (CA) enzymes; phenol content, *i.e.*, free phenols, conjugated phenols, and total phenols. Furthermore, a remarkable increase was recorded in the green pods chemical composition in protein and carbohydrates content (%) and Nitrogen (N), Phosphorus (P) and Potassium (K) minerals content compared to the untreated control.

Keywords: Bean; *Phaseolus vulgaris*; rust; *Uromyces appendiculatus*; biotic; abiotic inducers; systemic resistance; defense-related enzymes; total phenols.

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most consumed vegetable food legume worldwide either as green pods or as dry seeds and helps in improve fertility of the soil by biological N^2 fixation (Singh, 1999 and Al-Ballat and Al-Araby, 2019). In 2019, the global common bean harvested area was 33.1 million ha and produced 28.9 million tons (FAOSTAT, 2019). Whereas in Egypt, total cultivated area for snap bean in 2020/2021 growing season was 40988 feddan, which yielded 171375 tons, with a yield average 4.181 tons/feddan green bean, as well as the total cultivated area for dry bean (common bean) was 120676 feddan, which yielded 134591 tons, with a yield average 1.115 ton/feddan. as statistically given by Ministry of Agriculture and Land Reclamation, Egypt.

Bean (*Phaseolus vulgaris* L.) is liable to attack by several diseases which are responsible to cause considerable loss in the yield and its components. *Uromyces appendiculatus* Pers., is obligate biotrophic plant pathogen causing one of the most distribution and destructive diseases worldwide affecting the common bean yield allover production areas (Araya *et al*., 2004 and Souza *et al*., 2005). The disease under the favorable high relative humidity causes yield losses of 18 to 100% in crop quantity and quality across the world (Broughton *et al*., 2003 and Alexandratos and Bruinsma, 2012). In Egypt, bean rust *Uromyces appendiculatus* Pers. is widespread recording varied disease severity in the different governorates causing yield losses of dry and snap bean productions up to 70% (El-Hamady *et al*., 2010 and El-Fawy and Abo-Elyousr, 2016), whereas, Said and Taher (2020)

and Omara *et al*. (2022) studied the partial resistance (PR) to bean rust (*Uromyces appendiculatus*) through three epidemiological parameters, area under disease progress curve (AUDPC), final rust severity (FRS %), and rate of disease increase (r-value) and found that they were varied significantly among bean varieties tested under both field and greenhouse conditions in different years. Meanwhile, several *U. appendiculatus* physiological races based on virulence variability have been identified. Most plant diseases are controlled by fungicides. However, stimulation of resistance has become important as non-classical and eco-friendly alternatives have been used to avoid the use of pesticides to control plant diseases as they can safety affect environment and reduce damage of ecosystems have achieved this success (Reddy *et al*., 2014 and Ozkara *et al*., 2016).

The application of biotic or abiotic resistance inducers has positive effects that improve plant growth and eliminate indirect diseases. (Ozkara *et al.*, 2016; Prasannath, 2017 and Sarhan *et al*., 2018). For several years great effort has been devoted to the study of using biotic or abiotic agents in inducing systemic defense reaction including indole acetic acid (IAA), pathogenesis-related proteins, lignin synthesis, and defense-related enzymes such as peroxidase, polyphenoloxidase, phenylalanine ammonia-lyase, *β*-1,3-glucanase, and Catalase using Rhizobacteria (PGPR) in response to fungal infection of the host plants to management foliar plant diseases (Abd El-Rahman *et al.*, 2012; Sarhan *et al.*, 2018 and Yu *et al*., 2022)

Furthermore, foliar treatments by biotic or abiotic resistance inducers increased the phenolic compounds content, several investigators reported that role of phenolic compounds as antimicrobial substances against infection was by increasing the host cell wall mechanical strength (Nicholson and Hammerschmidt, 1992; Benhamou *et al.*, 2000 and Khaledi *et al.*, 2015).

The aim of this study was to evaluate the efficacy of some biotic and abiotic resistance inducers on rust in bean plants in *vitro* and in field conditions. In addition, the activity of protective enzymes and the phenolic compounds accumulation, and the growth and yield parameters of bean seeds were investigated.

MATERIALS AND METHODS

1- Source of bean seeds:

Bean (*Phaseolus vulgaris* L.) seeds cultivar, Paulista were kindly obtained from Department of Vegetable Crops Res.; Hortic. Res. Inst. (HRI); Agricultural Research Centre, Giza, Egypt.

2- Collection of rust inocula:

Uredospores of bean rust *Uromyces appendiculatus* Pers., were collected from infected bean plants showing rust symptoms, in Menoufia governorate, through February and March (during the growing season) 2018. The collected uredospores were identified according to Chung *et al*. (2004) based on their morphological description as *U. appendiculatus*. The uredospores were dried and stored under freezing conditions in microtubes for further experiments.

3- Purification of rust isolates:

Bean seeds (Paulista cv) were sown into sterilized pots (25 cm), already filled with autoclaved Nile silt sandy soil. Five replicate pots were used for each isolate and five seeds were sown per pot. Pots were kept under greenhouse conditions and irrigated when it was necessary. Two weeks later, seedlings were thinned into 4 plants.

Bean seedlings were inoculated using *Uromyces appendiculatus*, uredospores, at the first leaf, according to Stakman *et al*. (1962), by rubbing the leaves with moistened fingers after spraying with water. Uredospores were gently shaken over the plant leaves.

Inoculated plants were covered with moistened plastic cage and incubated for 24 hours in complete darkness and high humidity to enhance spore germination. Two days after inoculation, the plastic cover was loosened then pots were transferred to continuous high-output fluorescent lamps and high humidity under the greenhouse conditions. Plants were visually inspected 12 to 16 days after inoculation looking for the formation of uredial pustules. Isolates were purified using single pustule method (Green, 1981) and propagated on their original bean Paulista cv. under plastic cap to exclude cross contamination and to be used for further studies.

4- Source of used bioagents:

Fungal and bacterial bio-agents were sprayed on bean foliar to control bean rust disease. The tested *Trichoderma harzianum* and *Trichoderma viride* isolates were kindly obtained from Vegetable Diseases Research Department, Plant Pathology Research Institute, ARC, Giza, Egypt, as well the two bacteria species, *Bacillus subtilis* and *Paenibacillus polymyxa* were obtained from Soil, Water and Environment Research Institute (SWERI), Agricultural Research Centre, Giza, Egypt.

5- Source of the tested Chemical inducers:

Bion WG. 50%, Benzothiadiazole, was obtained from Syngenta Crop Protection, Inc., while both Salicylic acid and Potassium citrate were obtained from Sigma Aldrich, USA.

6- The tested fungicides:

Domark EC 10% & Tilt EC 25% fungicides (Table, 1) were used.

Table (1): Trade name, chemical name, active ingredient %, dosage of the tested fungicides.

Trade name	Chemical name	Active ingredient %	dosage/ 100 L.	Chemical name
Domark	Tetraconazole	EC 10%	50 mL	1H-1,2,4-Triazole, 1-(2-(2,4-dichlorophenyl)-3-(1,1,2,2-tetrafluoroethoxy) propyl
Tilt	Propiconazole	EC 25%	25 mL	1-((2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl) methyl)-1H-1,2,4-triazole

7. Preparation of the tested bioagents inocula:

7.1- Preparation the inocula of tested Fungal bioagents:

Preparation the inocula of *T. harzianum* and *T. viride* were prepared by culturing them in sterilized 500cc glass bottles containing 100 g of sorghum grain medium. (Rini and Sulochana, 2007). Each bottle was inoculated with four 7-day old cultures 0.5 cm diameter mycelial discs of the desired fungus. The bottles were incubated with vigorous shaking daily for 18 days at 27±1°C to promote rapid and efficient sorghum grains colonization. Sorghum grains colonized by mycelium and conidia of *T. harzianum* or *T. viride* were removed from the bottle, air-dried at room temperature in a cool place, then finely ground in a mill and passed through a sieve 60-mesh (0.25 mm) (Tewari and Bhanu, 2004). Then stored in sterile plastic bags at room temperature until use. The adjusted formula for *T. harzianum* and *T. viride* was 3×10^7 CFU/g (Sarhan *et al.*, 2018).

7.2- Preparation of the tested bacterial bioagents inocula:

Bacillus subtilis and *P. polymyxa* cultures were re-grown from fresh slants, transferred this to a flask containing 50 ml of (NYDB) medium (per liter: 8 g nutrient broth, yeast extract (5 g) and 10 g dextrose) and incubated at 24±1°C on 120 rpm rotary shaker for 3 days. The bacterial suspension concentration was adjusted using spectrophotometer with measuring absorbance at 600 nm (A600) and *B. subtilis* and/or *P. polymyxa* standard curves were adjusted to 5×10^8 CFU/mL (Sinclair and Dhingra, 2019).

8- *in vitro* effect of some biotic and abiotic factors on *Uromyces appendiculatus* uredospore germination:

The effect of some biotic and abiotic factors *i.e.*, Ascorbic acid, Bion, Salicylic acid, Potassium citrate, *B. subtilis*, *P. polymyxa*, *T. harzianum*, and *T. viride* as well as the fungicides, Tilt EC 25%and Domark EC 10% on germination of *U. appendiculatus* uredospore *in vitro* was evaluated.

The tested *T. harzianum* and *T. viride* isolates were grown at 25±2°C for 7-10 days on potato dextrose broth (PDB), The obtained spore suspension was adjusted as 10^8 spores/mL concentration using hemocytometer slide.

The tested bacterial bio-agents *i.e.*, *B. subtilis* and *P. polymyxa* suspensions were prepared as previously mentioned and the suspension concentration was adjusted to 5×10^8 CFU/mL (Sinclair and Dhingra, 2019).

U. appendiculatus uredospores were transferred to each tested fungal or bacterial concentration. In a sterilized Petri dish on sterilized slide (two slides in each) 1 mL of uredospore suspension was placed. Control treatment was only sterilized distilled water and spore suspension. The plate was supported with a cotton swab moistened with sterile distilled water to keep the high relative humidity. Three Petri dishes were used for each treatment and incubated at 24±1°C for 24 h. The uredospores germination percentage over control was calculated.

The four chemical factors *i.e.*, Ascorbic acid, Bion, Salicylic acid, and Potassium citrate as well as Tilt EC 25% and Domark EC 10% fungicides, were added to PDA medium with the recommended rates (Table 1). A volume of 50 µl of *U. appendiculatus* spore suspension (1×10^5 /mL spores) was placed directly onto PDA plates. then plates were placed in the dark for 24 h at 24±1°C. The uredospores germination percentage over control was calculated. The Inhibition of uredospores germination was calculated using the following equation:

$$\textbf{Inhibition \%} = \frac{\textbf{control-treatment}}{\textbf{control}} \times 100$$

9- Bean rust management under field conditions:

9.1- Field trials:

Field experiments to evaluate the tested biotic and abiotic resistance inducers effect for controlling bean rust under naturally field infection, with rust, at Ashmoun, Menoufia Governorate, Egypt, were carried out through both of 2019 and 2020 successive seasons in the field on 1st February 2019 and 2020. Field trial consisted of 33 plots, each plot area was 10.5 m^2, consisted of five rows (3.5 m length and 0.6 m width), on the western side of the row ridge, in 20 cm $hills^{-1}$ apart with two seeds/hill all treatments were planted by bean seeds cv. Paulista that were sown by three replicates in complete randomized block design. The other agricultural practices were based on the recommendation proposed by Horticulture Research Institute, ARC. Egypt.

The tested treatments: (1) *B. subtilis*, (2) *P. polymyxa*, (3) *T. harzianum*, (4) *T. viride*, (5) Ascorbic acid, (6) Bion, (7) Potassium citrate, (8) Salicylic acid, (9) Domark EC 10% and (10) Tilt EC 25%, (11) Control (bean plants sprayed with water served as untreated control).

The growing plants were sprayed three times at 25, 40, and 65 days after sowing with the tested bioagents, chemical resistance inducers and fungicides, during both growing seasons (2019 and 2020).

9.2- Disease Assessment:

The infection of bean rust, *U. appendiculatus* was assessed and estimated using four pathological parameters *i.e*., Rust severity (%), Area Under the Disease Progress Curve (AUDPC), Final rust severity, and r-value, as mentioned before. Each of these parameters was subjected to the analysis of different tested treatments to manage bean rust under natural field conditions.

Disease score "disease severity" data were recorded, 30 d. after sowing from the beginning of March weekly eight times to the end of April.

8-2- Rust severity (%):

Disease severity "rust severity" was subjected to assess the affected leaf area with rust infection according to the visual key prepared by Stonehouse (1994), as the symptoms were divided from 1-9 classes. For calculating disease severity% the following equation was used:

$$\textbf{\% Disease severity} = \frac{\textbf{Sum of (n} \times \textbf{v)}}{\textbf{9 N}} \times 100$$

Where:

n = number of plants in every grade.

v = numerical grade.

N = total number of examined leaflets.

9 = maximum disease grade.

Area Under Disease Progressive Curve (AUDPC) was calculated using the following equation of Pandey *et al*. (1989) to compare disease amounts among different treatments.

$$\textbf{AUDPC} = \textbf{D [1/2(Y1 + Yk) + Y2 +Y3 +… +Y (k-1)]}$$

Where:

D = days between readings

Y1 = first disease record

YK = last disease record.

The final rust severity was calculated as reported by Das *et al*. (1993) after the highly susceptible check cultivar disease severity (%) reaching the complete infection.

the rate of disease increase (r-value) was determined using the following Equation (Van der Plank, 1963):

$$\textbf{r-value} = \frac{1}{t_2 - t_1} \left(\log_e \frac{X_2}{1 - X_2} - \log_e \frac{X_1}{1 - X_1}\right)$$

Where:

X_1 = Disease severity (%) at t_1 date.

X_2 = Disease severity (%) at t_2 date.

$t_2 - t_1$ = Time interval in days between two observations.

9.3- Effect of spraying bean plants with the tested treatment on the bean crop and yield parameters:

Snap beans (cv. Paulista) crop and yield parameters in two successive seasons 2019 and 2020 were estimated. In the end of each season the average of some crop parameters, *i.e.*, number of branches/plant, plant height (cm), plant fresh and dry weight (g), were estimated, also the average of some yield parameters, *i.e.*, pod length (cm), average pod weight/plant (g) and yield (ton/fed) were taken into consideration.

9.4- Green pods chemical composition:

The collected proper maturity Snap bean green pods as described by Bremner and Malvaney (1983) were dried at 70°C and digested with H_2SO_4 and H_2O_2 until constant weight.

9.4.1- The green pods minerals content (N.P.K) assay:

Total nitrogen and Phosphorus were determined colorimetrically using a spectrophotometer according to Novozamsky *et al*. (1984) and Wilde *et al*. (1985), respectively, as well according to Black (1965), Potassium content using the flame photometer was determined.

9.4.2- Green pods Protein and Carbohydrates content assay:

Green pods were randomly collected in triplicates from plots and according to AOAC (2000), total carbohydrates, crude protein (% N × 6.25), crude fibers were determined, and the means were calculated.

9.5-Determination of photosynthetic pigments:

Bean leaf sample from the blade of the 3rd leaf from the tip was collected randomly, three replicates for each treatment to extract the chlorophyll a, b, and carotenoids (Photosynthetic pigments) according to (Robinson and Britz, 2000) from the 2019 and 2020 growing seasons and determined using the following equation (Spomer *et al.*, 1988).

Chlorophyll A (mg/g) =
(16.5×E665 - 8.3 × E650)/5.

Chlorophyll B (mg/g) =
(33.8×E650 - 12.5 × E665)/5.

Total Chlorophyll (mg/g) =
(25.5×E650 – 4 × E665)/5.

Carotenoids (mg/g) =
(4.2×E452.5 - 0.0264 × Chl.A - 0.496 × Chl.B)/5.

9.6- Effect of spraying bean plants with different treatments on the activity of defense related enzymes and phenol content:

The sprayed bean plants were sampled after 48 hours of last treatment and analyzed for Catalase (CA), Peroxidase (PO), polyphenoloxidase (PPO) enzymes activity and phenols contents were determined in bean plants tissue extracts representing the tested treatments: 1) *B. subtilis*, (2) *P. polymyxa*, (3) *T. harzianum*, (4) *T. viride*, (5) Ascorbic, (6) Bion, (7) Potassium citrate, (8) Salicylic acid, (9) Domark EC 10% and (10) Tilt EC 25%, and (11) Control. All treatments were grown under natural field infection.

Shoot samples 48 hours after the last treatment were collected and also the untreated healthy control treatments. Plant tissue (1 g) was prepared according to Biles and Martyn, (1993) and the Catalase (CA), Peroxidase (PO), and polyphenoloxidase (PPO) activities were determined according to Soltis and Soltis (1998). Each treatment consisted of three replicates (three plants/replicate), and spectrophotometric readings were performed two times per replicate using a Milton Roy 1201 (PEMEDR, Denver, CO, USA) Spectrophotometer.

9.6.1- Catalase (CAT) assay:

Activity of Catalase was determined at 240 nm (Σ= 39.4 mM-1 cm-1) Spectrophotometrically for 3 min. by measuring H_2O_2 consumption. using Dhindsa *et al*. (1981) method, as units/mg protein/min the CAT activity was expressed.

9.6.2- Peroxidase (PO) assay:

Peroxidase (PO) activity was determined using the method recorded by Hammerschmidt *et al.* (1982) spectrophotometrically at 470 nm. for 1 min. as PO units/mg protein/min the CAT activity was expressed (Urbanek *et al.*, 1991).

9.6.3- Polyphenol oxidase (PPO) assay:

The activity of PPO was determined spectrophotometrically according to Gauillard *et al.* (1993) was determined of absorbance at 410 nm during 10 min at 30°C, the activity was expressed as PPO units/mg protein/min.

9.6.4- Determination of phenolic compounds:

Total phenol assay was spectrophotometrically according to Agbor *et al.* (2014). in a UV spectrophotometer by the absorbance read at 750 nm.

10- Statistical analysis:

Data were subjected to one-way ANOVA as described by Gomez and Gomez (1984). An automatic least significant difference (LSD) test was performed to compare treatment means with a probability of 5%.

RESULTS

Effect of some biotic, abiotic factors and fungicides on uredospore germination of *U. appendiculatus in vitro*:

Data illustrated in Table (2) show the inhibitory effect of the tested antagonistic bio-agents and chemical inducers of resistance as well as two fungicides on germination of *U. appendiculatus* uredospores *in vitro*.

All the tested bio-agents and chemical inducers of resistance as well as two fungicides significantly inhibited uredospores germination compared with control treatment. Results in Table (2) show that Domark EC 10% fungicide was the most effective treatment followed by Tilt EC 25% fungicide without significant deference followed by *B. subtilis*, where they recorded 2.7, 3.3 and 16.0% of germination and 97.1, 96.3 and 82.4% of inhibition, respectively. Meantime, *T. harzianum*, *T. viride*, *P. polymyxa*, Salicylic acid, Bion, Potassium citrate and Ascorbic acid, recorded 18.3, 20.3, 24.0, 27.3, 30.0, 33.3 and 38.0 germination% as well as percentages of inhibition were 79.9, 77.7, 73.6, 70.0, 67.0, 63.4 and 58.2% respectively, compared with the control, being 91.0% germination.

Table (2): Effect of some biotic, abiotic resistance inducers, and fungicides *in vitro* on *U. appendiculatus* uredospores germination

Treatment	Germination %	Inhibition %
Bacillus subtilis	16.0 g	82.4
Paenibacillus polymyxa	24.0 e	73.6
Trichoderma harzianum	18.3 fg	79.9
Trichoderma viride	20.3 f	77.7
Ascorbic acid	38.0 b	58.2
Bion	30.0 d	67.0
Potassium citrate	33.3 c	63.4
Salicylic acid	27.3 d	70.0
Tilt EC 25%	3.3 h	96.3
Domark EC 10%	2.7 h	97.1
Control	91.0 a	-
LSD at 0.05	3.67	-

Management of bean rust under field conditions:

This experiment was carried out through 2019 and 2020 successive growing seasons in Ashmoun (Menoufia governorate), to evaluate the effect of the tested biotic and abiotic resistance inducers for management bean rust (*U. appendiculatus*) under field conditions. Bean cv. Paulista was used in this experiment.

As shown in Figs. (1 and 2) spraying of bean plants under field conditions with all the tested biotic and abiotic resistance inducers *i.e*., *Bacillus subtilis*, *Paenibacillus polymyxa*, *Trichoderma harzianum*, *Trichoderma viride*, Ascorbic acid, Bion, Potassium citrate, Salicylic acid, as well as the two tested fungicides *i.e*., Domark EC 10% and Tilt EC 25%, significantly reduced rust severity, AUDPC and r-value during the two successive growing seasons (2019 and 2020) compared to the control treatment.

Data in Figs. (1 and 2) show the efficiency of the ten tested treatments in controlling rust of bean under field conditions during the growing season of 2019. Tilt EC 25% and Domark EC 10% fungicides significantly gave the highest reduction effect on rust severity and (AUDPC) being 134.4 and 160.1 AUDPC with 6.0 and 6.7% final rust severity, compared to the untreated control treatment1 (978.7 AUDPC and 90.7%) and final rust severity, respectively, followed by Bion, *T. harzianum*, *B. subtilis*, Salicylic acid, *P. polymyxa* and *T. viride*, being 214.7, 240.3, 267.2, 295.2, 326.7 and 406.0 AUDPC, and with 9.0, 9.7, 11.7, 13.0, 13.7 and 20.3% final rust severity, respectively, While Ascorbic acid, and Potassium citrate, being 471.3 and 500.5 AUDPC, and with 23.3 an 24.3% final rust severity showed the lowest reduction, respectively.

Also, all treatments have significantly decreased the disease increase rate (r-value) during the 2019 season, being 0.047, 0.046, 0.048, 0.055, 0.055, 0.047, 0.056, 0.049, 0.059 and 0.056 for *B. subtilis*, *P. polymyxa*, *T. harzianum*, *T. viride*, Ascorbic acid, Bion, Potassium citrate, Salicylic acid, Domark EC 10% and Tilt EC 25%, respectively, compared to the control 0.096.

Data in Figs. (1 and 2) confirmed the efficiency of ten tested treatments in controlling bean rust during the growing season 2020 as the obtained data showed the same trend of the first growing season of 2019. The fungicides Tilt EC 25% and Domark EC 10% significantly gave the highest reduction effect on rust severity and (AUDPC), being 120.4 and 147.2 AUDPC with 5.3 and 6.3% final rust severity, compared to the untreated control treatment1 (1568.0 AUDPC and 75.0%) final rust severity, respectively, following by Bion, *T. harzianum*, *B. subtilis*, Salicylic acid, *P. polymyxa* and *T. viride*, being195.0, 217.0, 252.0, 276.6, 302.2 and 368.8 AUDPC, and with 8.3, 9.0, 11.0, 12.3, 13.3 and 18.3 final rust severity respectively, Whereas the lowest effect was attributed to Ascorbic acid, and Potassium citrate, being 430.5 and 464.3 AUDPC, and with 21.0 and 22.0% final rust severity respectively.

Also, all treatments significantly decreased the rate of disease increase (r-value) during the growing season 2019, being 0.047, 0.046, 0.048, 0.055, 0.055, 0.047, 0.056, 0.049, 0.059 and 0.056 for *B. subtilis*, *P. polymyxa*, *T. harzianum*, *T. viride*, Ascorbic acid, Bion, Potassium citrate, Salicylic acid, Domark EC 10% and Tilt EC 25%, respectively, compared to the untreated control treatment 0.096. On the other hand, there were not significant differences among the values of decreasing the (r-value) during the growing season 2020, being 0.063, 0.062, 0.061, 0.061, 0.058, 0.056, 0.054, 0.052, 0.051 and 0.047 for *T. viride*, Potassium citrate, Ascorbic acid, Salicylic acid, Domark EC 10%, *P. polymyxa*, Tilt EC 25%, Bion, *B. subtilis*, and *T. harzianum*, respectively, compared to the untreated control treatment, 0.086.

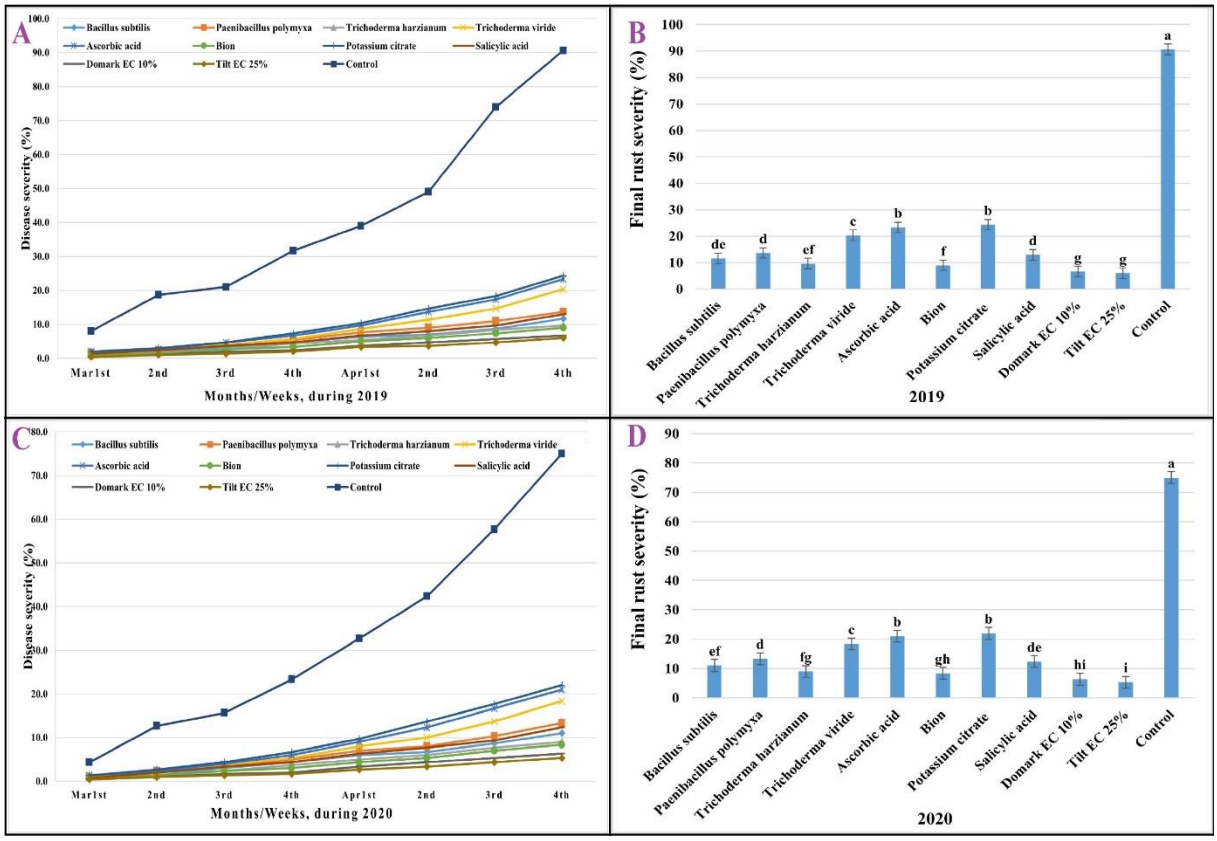

Figure (1): Effect of some biotic, abiotic factors and fungicides on percentage of bean rust (*U. appendiculatus*) severity (A&C) and final rust severity (B&D) of bean C.V. Paulista under field conditions during the growing seasons 2019 and 2020.

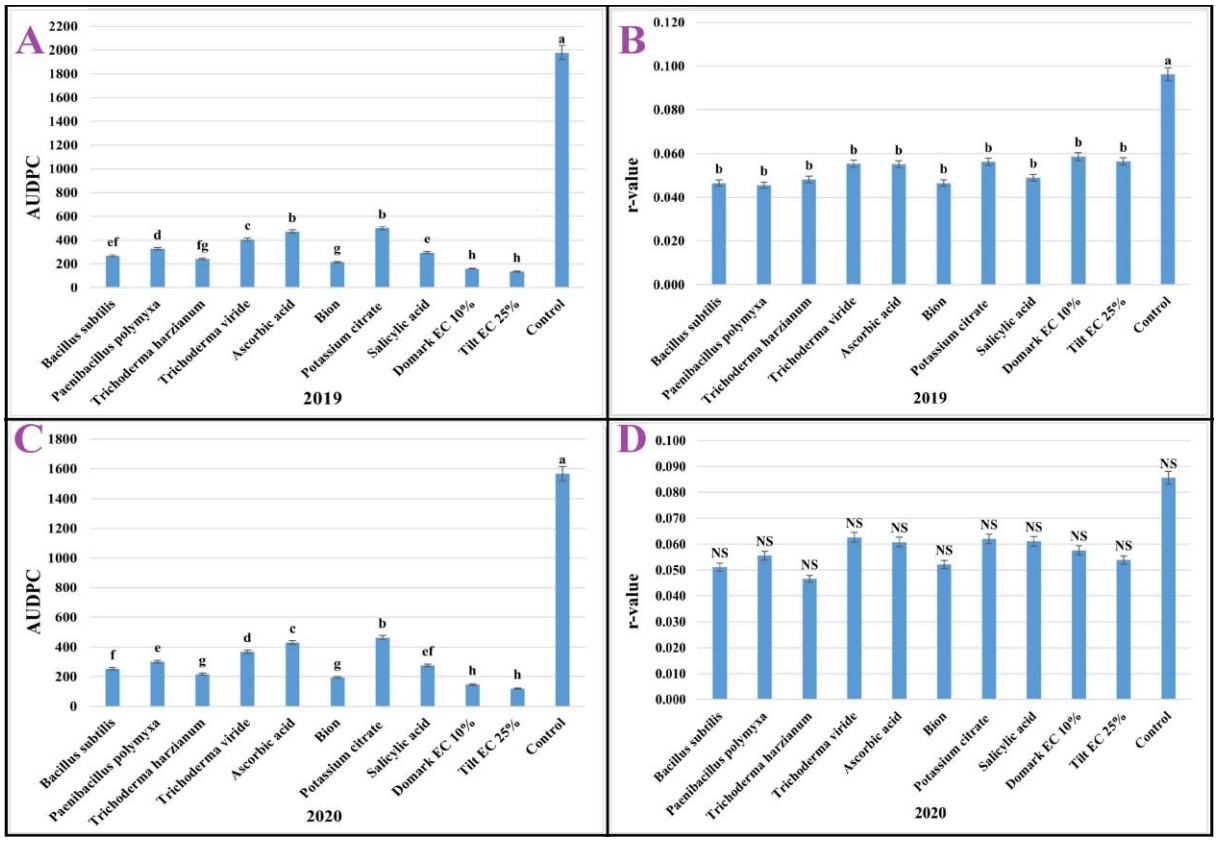

Figure (2): Effect of some biotic, abiotic factors and fungicides on AUDPC (A&C) and r-value (B&D) of bean rust (*U. appendiculatus*) of bean C.V. Paulista under field conditions during the growing seasons 2019and 2020.

Effect of some biotic, abiotic factors and fungicides on snap bean growth parameters:

Data in Table (3) reveal that growth parameters *i.e.*, plant height, No. of branches/plant, plant fresh and dry weight performances of beans (cv. Paulista) were significantly increased in all treatments as compared with untreated control in both 2019 and 2020 growing seasons.

Bion and Domark EC 10% recorded the maximum values of plant height, No. of branches/plant, plant fresh and dry weight, (50.90 cm, 5.87, 88.25 g and 17.25 g) and (49.33 cm, 6.10, 86.90 g and 16.72g), respectively, in 2019 and 2020 growing seasons, compared with untreated control (36.47 cm, 3.35, 69.08 g and 14.33 g), followed by Tilt EC 25% (48.42 cm), *P. polymyxa* (46.32 cm), *T. harzianum* (45.82 cm), *B. subtilis* (45.17 cm), Salicylic acid (44.72 cm), *T. viride* (42.37 cm), for the plant heigh respectively, Tilt EC 25% (5.48), *T. harzianum* (5.42), *B. subtilis* (5.05), *P. polymyxa* (4.97), Salicylic acid (4.78,) and *T. viride* (4.52), for No. of branches / plant respectively, *P. polymyxa* (85.77 g), *B. subtilis* (83.92 g), *T. harzianum* (83.88 g), Tilt EC 25% (83.37 g), *T. viride* (82.67 g) and Salicylic acid (82.57 g), for Plant fresh weight (g) respectively, *B. subtilis* (16.88 g), *P. polymyxa* (16.75 g), Tilt EC 25% (16.23 g), Salicylic acid (15.98 g), *T. harzianum* (15.77 g) and *T. viride* (15.72 g) for Plant dry weight (g) respectively.

Meantime, the Ascorbic acid and Potassium citrate recorded the lowest values of plant height, No. of branches/plant, plant fresh and dry weight, (41.15 cm, 4.07, 81.27 g and 15.42 g) and (40.80 cm, 4.12, 79.90 g and 14.97 g), respectively, compared with untreated control (36.47 cm, 3.35, 69.08 g and 14.33 g). However, the obtained values during the first season (2019), were significantly increased than those of the second 2020 season, while there were no significant differences between the two growing seasons on the average.

Effect of some biotic, abiotic factors and fungicides on snap bean yield parameters:

Data in Table (4) show that all treatments significantly increased beans (cv. Paulista) performances of yield parameters *i.e.*, pod length (cm), average pod weight/plant (g) and yield (ton/fed) as compared with untreated control in the two seasons 2019 and 2020.

Bion, Domark EC 10% and Tilt EC 25% recorded the highest values of pod length (cm), average pod weight/plant (g) and yield (ton/fed), (14.47 cm, 46.67 g and 4.43 ton/fed), (14.07 cm, 45.67 g and 4.00 ton/fed) and (13.77 cm, 44.50 g and 3.92 ton/fed), respectively, in 2019 and 2020 seasons, compared with untreated control (8.40 cm, 30.17 g and 2.43 ton/fed), followed by *B. subtilis* (13.23 cm), *P. polymyxa* (13.15 cm), *T. harzianum* (12.87 cm), Salicylic acid (12.65 cm) and *T. viride* (12.20 cm) for the pod length (cm), respectively. In case of, determined average weight of pods, *B. subtilis* (4.42 g), *P. polymyxa* (4.40 g), Salicylic acid (4.30 g), *T. viride* (4.23 g) and *T. harzianum* (4.18 g) for average pod weight/plant (g) respectively, *T. harzianum* (3.92 ton/fed), *P. polymyxa* (3.90 ton/fed), *B. subtilis* (3.80 ton/fed), Salicylic acid (3.72 ton/fed) and *T. viride* (3.55 ton/fed). Meantime, Ascorbic acid and Potassium citrate recorded the lowest values of pod length (cm), average pod weight/plant (g) and yield (ton/fed), (11.53 cm, 4.17 g and 3.37 ton/fed) and (10.73 cm, 4.07 g and 3.18 ton/fed), respectively, compared to untreated control (8.40 cm, 3.02 g and 2.43 ton/fed). However, in the 2019 season, all growth parameters values were increased significantly than those in the second growing season 2020.

Effect of snap bean treatments with some biotic, abiotic resistance inducers and fungicides on Photosynthetic pigments of bean plants:

Data presented in Table (5) show that significant increase in chlorophyll A, B, total chlorophyll and carotenoids (photosynthetic pigments) was resulted due to spraying snap bean plants with the tested biotic or abiotic inducers comparing to the control, in both2019 and 2020 growing seasons, indicated good reflecting of the plant vigor due to the application of chemicals or biotic inducers.

Bion and Domark EC 10%, treatments recorded the highest averages of values of photosynthetic pigments in the two successive growing seasons 2019 and 2020, being 1.544, 0.938 and 1.241 mg/g fresh weight and 1.493, 0.891 and 1.192 mg/g fresh weight compared with untreated control 1.211, 0.666 and 0.939 mg/g fresh weight, respectively, in case of chlorophyll A, it was followed by *T. harzianum*, Tilt EC 25%, *B. subtilis*, Salicylic acid, *P. polymyxa*, *T. viride*, Ascorbic acid and Potassium citrate, being (1.467, 1.425, 1.384, 1.362, 1.323, 1.301, 1.282 and 1.261 mg/g fresh weight) respectively, meantime in case of chlorophyll B, followed by Tilt EC 25%, *T. harzianum*, *B. subtilis*, Salicylic acid, *P. polymyxa*, *T. viride*, Ascorbic acid and Potassium citrate, being (0.856, 0.832, 0.808, 0.793, 0.773, 0.761 and 0.741 mg/g fresh

weight), respectively. Meanwhile, total chlorophyll was followed by *T. harzianum*, Tilt EC 25%, *B. subtilis*, Salicylic acid, *P. polymyxa*, *T. viride*, Ascorbic acid and Potassium citrate, being (1.150, 1.141, 1.096, 1.077, 1.048, 1.031, 1.011 and 0.991 mg/g fresh weight) respectively.

As well as, in case of carotenoids, the two tested fungicides, Domark EC 10% and Tilt EC 25% recorded the highest values of carotenoids in both 2019 and 2020 growing seasons, being (0.378 and 0.326 mg/g fresh weight) compared with untreated control (0.213 mg/g fresh weight) Following by *T. harzianum*, Bion, *B. subtilis*, *P. polymyxa*, *T. viride*, Salicylic acid, Potassium citrate and Ascorbic acid being (0.306, 0.305, 0.261, 0.253, 0.250, 0.248, 0.244 and 0.220 mg/g fresh weight), respectively.

However, in 2019 season, all the determined photosynthetic pigments were significantly increased compared to those recorded in 2020 season, while there were no significant differences between the two seasons.

Effect of spraying snap bean plants with some biotic, abiotic resistance inducers and fungicides on the oxidative enzymes activity:

Activities of peroxidase (PO), polyphenol oxidase (PPO) and catalase (CA) enzymes of snap bean plants were evaluated in the presence of *U. appendiculatus* (Table, 6). Results show a significant increase in enzyme activities. The highest value and increase of peroxidase (PO) activity was achieved with Bion treatment, being (0.372 unit/mg protein/min) by 115.2% increase compared to control (0.173 unit/mg protein/min), followed by *T. harzianum*, *B. subtilis*, Salicylic acid, and *P. polymyxa*, being 0.361, 0.335, 0.306 and 0.282 unit/mg protein/min and by 109, 94, 77 and 63% increase over untreated control, meantime, *T. viride*, Ascorbic acid, Tilt EC 25%, Potassium citrate and Domark EC 10% showed a considerable increase being 0.265, 0.248, 0.234, 0.220 and 0.209 unit/mg protein/min and by 53, 43, 35, 27 and 21% increase over control.

In case of polyphenol-oxidase (PPO), *T. harzianum* treatment recorded the highest value and increase of polyphenol oxidase (PPO) activity being (0.272 unit/mg protein/min) by 143.9% increase over untreated control (0.112 unit/mg protein/min), followed by *B. subtilis*, Bion, *P. polymyxa* and Salicylic acid being (0.261, 0.235, 0.206 and 0.182 unit/mg protein/min) and by 134.0, 110.7, 84.5 and 63.0% increase over untreated control, respectively, as well as, *T. viride*, Tilt EC 25% and Domark EC 10% treatments showed a considerable activity of polyphenol oxidase (PPO) being (0.165, 0.148 and 0.134 unit/mg protein/min), by 48.1, 32.2 and 19.7% increase over untreated control respectively, Meanwhile, Ascorbic acid and Potassium citrate treatments were not able to cause significant increase in polyphenol oxidase (PPO) activity compared to the untreated control (0.173 unit/mg protein/min), being (0.120 and 0.112 unit/mg protein/min) by 7.5 and 0.6% increase over untreated control, respectively

In case of catalase (CA) Bion treatment recorded the highest value and increase of catalase (CA) activity, being (0.173 unit/mg protein/min) by 52.4% increase over untreated control (0.113 unit/mg protein/min), Table (6) followed by *B. subtilis*, *T. harzianum* and *T. viride* which recorded (0.171, 0.168 and 0.166 unit/mg protein/min) and by 50.9, 48.2 and 46.5% increase over untreated control, respectively, as well as, Salicylic acid, Ascorbic acid and *P. polymyxa* treatments showed considerable percentages of catalase (CA) activity being (0.155, 0.145 and 0.138 unit/mg protein/min), by 36.5, 27.9 and 21.5% increase over untreated control, respectively. Meanwhile, the two tested fungicides Tilt EC 25% and Domark EC 10% and Potassium citrate did not cause significant increase in catalase activity compared to the untreated control (0.113 unit/mg protein/min) as they recorded (0.120, 0.118 and 0.117 unit/mg protein/min) by 5.6, 4.4 and 3.2% increase over untreated control, respectively.

Effect of spraying snap bean plants with some biotic, abiotic resistance inducers and fungicides on phenol content:

Total phenols coincided the same trend of the results of peroxidase (PO) polyphenol oxidase (PPO) and catalase (CA) enzymes, where it was highly enhanced in the sprayed snap bean plants compared to control plants in the presence of *U. appendiculatus* (Table, 7). The free phenolic compounds were highly increased with Bion treatment, being (6.29 mg/g fresh weight and 191.20% Increase over control), followed by *T. harzianum*, *B. subtilis*, Salicylic acid, Ascorbic acid, *P. polymyxa*, *T. viride* and Domark EC 10% treatments, the corresponding values were 6.13, 5.91, 5.72, 5.67, 5.23, 4.92 and 4.67 mg/g fresh weight, by 183.80, 173.61, 164.81, 162.50, 142.13, 127.78 and 116.20% increase over untreated control, whereas the least were recorded with Potassium citrate and Tilt EC 25%, being 4.31 and 3.88 mg/g fresh weight, by 99.54 and 79.63% increase over untreated control (2.16 mg/g fresh weight), respectively.

Table (3): Effect of some biotic, abiotic resistance inducers and fungicides on some plant growth parameters of snap bean (cv. Paulista) in 2019 and 2020 growing seasons.

Treatments	Plant height (cm)			No. of branches / plant			Plant fresh weight (g)			Plant dry weight (g)		
	*2019	2020	Mean	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
Bacillus subtilis	42.53	47.80	45.17	4.90	5.20	5.05	86.73	81.10	83.92	17.63	16.13	16.88
Paenibacillus polymyxa	46.70	45.93	46.32	5.13	4.80	4.97	90.43	81.10	85.77	17.77	15.73	16.75
Trichoderma harzianum	46.23	45.40	45.82	5.40	5.43	5.42	86.83	80.93	83.88	16.70	14.83	15.77
Trichoderma viride	43.83	40.90	42.37	4.43	4.60	4.52	85.70	79.63	82.67	16.30	15.13	15.72
Ascorbic acid	41.80	40.50	41.15	4.00	4.13	4.07	84.00	78.53	81.27	16.00	14.83	15.42
Bion	51.40	50.40	50.90	5.93	5.80	5.87	91.33	85.17	88.25	18.30	16.20	17.25
Potassium citrate	40.70	40.90	40.80	4.00	4.23	4.12	83.00	76.80	79.90	15.43	14.50	14.97
Salicylic acid	45.50	43.93	44.72	4.73	4.83	4.78	85.23	79.90	82.57	16.57	15.40	15.98
Domark EC 10%	49.87	48.80	49.33	5.80	6.40	6.10	90.20	83.60	86.90	17.43	16.00	16.72
Tilt EC 25%	48.90	47.93	48.42	5.53	5.43	5.48	86.80	79.93	83.37	17.00	15.47	16.23
Control	36.33	36.60	36.47	3.30	3.40	3.35	72.53	65.63	69.08	14.73	13.93	14.33
L.S.D. at 0.05												
Treatments (T)			1.50			0.242			1.560			0.791
Seasons (S)			0.64			0.103			0.665			0.337
T x S			2.12			0.343			2.206			1.118

* Growing season

Table (4): Effect of some biotic, abiotic resistance inducers and fungicides on snap bean (cv. Paulista) on yield parameters in 2019 and 2020 growing seasons.

Treatments	Pod length (cm)			Average pods weight/plant (g)			Yield (ton/fed)		
	*2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
Bacillus subtilis	13.13	13.33	13.23	4.50	4.33	4.42	3.90	3.70	3.80
Paenibacillus polymyxa	13.30	13.00	13.15	4.43	4.37	4.40	4.00	3.80	3.90
Trichoderma harzianum	12.93	12.80	12.87	4.00	4.37	4.18	4.03	3.80	3.92
Trichoderma viride	12.30	12.10	12.20	4.27	4.20	4.23	3.67	3.43	3.55
Ascorbic acid	11.73	11.33	11.53	4.23	4.10	4.17	3.50	3.23	3.37
Bion	14.23	14.70	14.47	4.63	4.70	4.67	4.70	4.17	4.43
Potassium citrate	10.73	10.73	10.73	4.23	3.90	4.07	3.27	3.10	3.18
Salicylic acid	12.70	12.60	12.65	4.30	4.30	4.30	3.80	3.63	3.72
Domark EC 10%	14.10	14.03	14.07	4.63	4.50	4.57	4.23	3.77	4.00
Tilt EC 25%	13.83	13.70	13.77	4.50	4.40	4.45	4.13	3.70	3.92
Control	8.10	8.70	8.40	3.30	2.73	3.02	2.43	2.43	2.43
L.S.D. at 0.05									
Treatments (T)			0.445			0.537			0.201
Seasons (S)			0.190			0.229			0.086
T × S			0.630			0.759			0.285

* Growing season

Table (5): Effect of some biotic, abiotic resistance inducers and fungicides on photosynthetic pigments of snap bean plants (cv. Paulista) under field conditions, during growing seasons 2019 and 2020.

Treatments	Chlorophyll A (mg/g fresh weight)			Chlorophyll B (mg/g fresh weight)			Total chlorophyll (mg/g fresh weight)			Carotenoids (mg/g fresh weight)		
	*2019	2020	Mean	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
Bacillus subtilis	1.414	1.354	1.384	0.820	0.795	0.808	1.117	1.074	1.096	0.265	0.256	0.261
Paenibacillus polymyxa	1.344	1.302	1.323	0.785	0.760	0.773	1.065	1.031	1.048	0.257	0.248	0.253
Trichoderma harzianum	1.490	1.444	1.467	0.846	0.819	0.832	1.168	1.131	1.150	0.311	0.301	0.306
Trichoderma viride	1.321	1.281	1.301	0.773	0.749	0.761	1.047	1.015	1.031	0.255	0.246	0.250
Ascorbic acid	1.302	1.262	1.282	0.754	0.727	0.741	1.028	0.994	1.011	0.223	0.218	0.220
Bion	1.568	1.519	1.544	0.953	0.922	0.938	1.261	1.221	1.241	0.309	0.301	0.305
Potassium citrate	1.276	1.245	1.261	0.724	0.718	0.721	1.000	0.982	0.991	0.248	0.241	0.244
Salicylic acid	1.384	1.340	1.362	0.805	0.780	0.793	1.095	1.060	1.077	0.251	0.244	0.248
Domark EC 10%	1.517	1.469	1.493	0.906	0.877	0.891	1.211	1.173	1.192	0.383	0.372	0.378
Tilt EC 25%	1.448	1.402	1.425	0.870	0.843	0.856	1.159	1.123	1.141	0.331	0.321	0.326
Control	1.229	1.193	1.211	0.676	0.656	0.666	0.953	0.925	0.939	0.215	0.211	0.213
L.S.D. at 0.05												
Treatment (T)			0.012			0.011			0.010			0.051
Seasons (S)			0.005			0.005			0.004			0.022
T × S			0.018			0.015			0.015			0.072

* Growing season

As for conjugated phenols, *B. subtilis* treatment recorded the highest increase, being 1.80 mg/g fresh weight, by 122.22% over the control 0.81 mg/g fresh weight, followed by Bion, *T. harzianum*, Salicylic acid, Ascorbic acid, *P. polymyxa*, *T. viride* and Domark EC 10%, being 1.76, 1.67, 1.65, 1.61, 1.54, 1.42 and 1.31 mg/g fresh weight, by 106.17, 103.70, 98.77, 90.12, 75.31 and 61.73% increase over the untreated control, whereas the minimum increases were recorded when Potassium citrate and Tilt EC 25% were applied, being 1.25 and 1.13 mg/g fresh weight, by 54.32 and 39.51% increase over untreated control, respectively.

Total phenolic content maximum increase was recorded with Bion, being 8.05 mg/g fresh weight, by 171.04% over the control 2.97 mg/g fresh weight, followed by *T. harzianum*, *B. subtilis*, Salicylic acid, Ascorbic acid, *P. polymyxa*, *T. viride*, Domark EC 10%, Potassium citrate and Tilt EC 25%, being 7.80, 7.71, 7.37, 7.28, 6.77, 6.34, 5.98, 5.56 and 5.01 mg/g fresh weight, by 159.60, 148.15, 145.12, 127.95, 113.47, 101.35, 87.21 and 68.69 increase over untreated control respectively.

Table (6): Effect of some biotic, abiotic resistance inducers and fungicides on peroxidase, polyphenoloxidase and catalase activities in treated snap bean plants (cv. Paulista) grown under field conditions and naturally infected by *U. appendiculatus*.

Treatment	Peroxidase (unit/mg protein/min)		Polyphenol oxidase (unit/mg protein/min)		Catalase (unit/mg protein/min)	
	Activity	Increase over control %	Activity	Increase over control %	Activity	Increase over control %
Bacillus subtilis	0.335 b	93.8	0.272 a	143.9	0.168 a	48.2
Paenibacillus polymyxa	0.282 d	63.0	0.165 d	48.1	0.166 a	46.5
Trichoderma harzianum	0.361 a	108.9	0.261 a	134.0	0.171 a	50.9
Trichoderma viride	0.265 d	53.4	0.206 c	84.5	0.138 d	21.5
Ascorbic acid	0.248 e	43.2	0.120 fg	7.5	0.145 c	27.9
Bion	0.372 a	115.2	0.235 b	110.7	0.173 a	52.4
Potassium citrate	0.220 fg	27.2	0.112 g	0.6	0.117 e	3.2
Salicylic acid	0.306 c	76.9	0.182 d	63.0	0.155 b	36.5
Tilt EC 25%	0.234 ef	35.1	0.134 ef	19.7	0.118 e	4.4
Domark EC 10%	0.209 g	21.0	0.148 e	32.2	0.120 e	5.6
Control	0.173 h		0.112 g		0.113 e	
LSD at 0.05	0.017		0.017		0.007	

Table (7): Effect of biotic, abiotic resistance inducers and fungicides on phenolic content levels in snap bean plants (cv. Paulista) grown under field conditions and naturally infected by *U. appendiculatus*.

Treatment	Phenolic contents (mg/g fresh weight)					
	Free phenols		Conjugated phenols		Total phenols	
	Mean	Increase over control %	Mean	Increase over control %	Mean	Increase over control %
Bacillus subtilis	5.91 c	173.61	1.80 a	122.22	7.71 b	159.60
Paenibacillus polymyxa	5.23 e	142.13	1.54 c	90.12	6.77 d	127.95
Trichoderma harzianum	6.13 b	183.80	1.67 b	106.17	7.80 b	162.63
Trichoderma viride	4.92 f	127.78	1.42 d	75.31	6.34 e	113.47
Ascorbic acid	5.67 d	162.50	1.61 b	98.77	7.28 c	145.12
Bion	6.29 a	191.20	1.76 a	117.28	8.05 a	171.04
Salicylic acid	5.72 d	164.81	1.65 b	103.70	7.37 c	148.15
Potassium citrate	4.31 h	99.54	1.25 e	54.32	5.56 g	87.21
Domark EC 10%	4.67 g	116.20	1.31 e	61.73	5.98 f	101.35
Tilt EC 25%	3.88 i	79.63	1.13 f	39.51	5.01 h	68.69
Control	2.16 j		0.81 g		2.97 i	
L.S.D. at 0.05	0.11		0.06		0.14	

Effect of some biotic or abiotic resistance inducers on protein and carbohydrate contents in green pods of snap bean:

Data presented in Table (8) show that snap bean green pods protein and carbohydrate contents (%) were significantly increased by all treatments in 2019 and 2020 growing seasons comparing with control.

Bion and *T. harzianum* treatments recorded the maximum protein content values (4.82 and 3.93%), followed by Domark EC 10%, Tilt EC 25%, *P. polymyxa*, Salicylic acid, *B. subtilis* and *T. viride*, being (3.87%, 3.75, 3.42, 3.40, 3.25 and 3.18%), Whereas Ascorbic acid and Potassium citrate recorded the lowest (3.02 and 2.83%) compared with untreated control (2.12%) in 2019 and 2020 seasons on the average, respectively. The same, regarding the carbohydrates content, Bion and *T. harzianum* recorded the maximum carbohydrates content (17.02 and 16.82%) followed by Salicylic acid, Domark EC 10%, Tilt EC 25%, *B. subtilis*, *P. polymyxa*, Salicylic acid and *T. viride*, recorded (16.48, 16.45, 16.00, 16.00, 15.87 and 15.53%, respectively, whereas Ascorbic acid and Potassium citrate recorded the lowest protein and carbohydrates (15.28 and 15.17%) compared with the control treatment in both growing seasons on the average, respectively.

Table (8): Effect of some biotic, abiotic resistance inducers and fungicides spray on snap bean (cv. Paulista) plants on the protein and carbohydrates content (%) of green pods during both 2019 and 2020 seasons

Treatments	Protein content in green pods (%)			Carbohydrates content (%) as dry weight		
	2019	2020	Mean	2019	2020	Mean
Bacillus subtilis	3.30	3.20	3.25	16.70	15.30	16.00
Paenibacillus polymyxa	3.50	3.33	3.42	16.60	15.40	16.00
Trichoderma harzianum	4.00	3.87	3.93	17.60	16.03	16.82
Trichoderma viride	3.20	3.17	3.18	16.03	15.03	15.53
Ascorbic acid	3.10	2.93	3.02	15.83	14.73	15.28
Bion	4.83	4.80	4.82	17.83	16.20	17.02
Potassium citrate	2.97	2.70	2.83	15.73	14.60	15.17
Salicylic acid	3.43	3.37	3.40	16.43	15.30	15.87
Domark EC 10%	4.03	3.70	3.87	17.13	15.83	16.48
Tilt EC 25%	3.90	3.60	3.75	17.00	15.90	16.45
Control	2.23	2.00	2.12	13.50	11.83	12.67
L.S.D. at 0.05						
Treatments (T)			0.11			0.44
Seasons (S)			0.05			0.19
T × S			0.15			0.62

Effect of some biotic, abiotic resistance inducers and fungicides on snap bean green pods minerals (N.P.K) content:

Data in Table (9) show that all treatments significantly increased minerals content, *i.e*., nitrogen, phosphorus, and potassium (N, P, K) of snap bean (cv. Paulista) green pods in the two growing seasons 2019 and 2020 compared with untreated control.

Bion and Domark EC 10% recorded the maximum of nitrogen (N) content values, being (3.22 and 3.07%), respectively, in both 2019 and 2020 seasons, compared with untreated control (1.57%), followed by *T. harzianum* (2.77%), *B. subtilis* (2.70%), Tilt EC 25% (2.68), *P. polymyxa* (2.62%), Salicylic acid (2.57%), *T. viride* (2.42%), Ascorbic acid (2.13%) and Potassium citrate (2.07%), respectively. In case of phosphorus (P) content, also Bion and the fungicide Domark EC 10% recorded the maximum phosphorus (P) content values, being (0.40 and 0.38%), respectively, in the two growing seasons 2019 and 2020, compared with untreated control (0.20%), followed by Salicylic acid (0.36%), *T. harzianum* (0.33%), *P. polymyxa* (0.32%), *T. viride* (0.32%), *B. subtilis* (0.30%), Ascorbic acid (0.28%), Tilt EC 25% (0.28%) and Potassium citrate (0.24%), respectively. In case of potassium (K) content, also Bion recorded the maximum potassium (K) content value, being (2.50%) in the two growing seasons 2019 and 2020, compared with untreated control (1.66%), followed by Salicylic acid (2.37%), *P. polymyxa* (2.35%), Domark EC 10% (2.31%), *B. subtilis* (2.28%), *T. viride* (2.27%), *T. harzianum* (2.23%), Tilt EC 25% (2.18%), Ascorbic acid (2.10%) and Potassium citrate (1.87%), respectively. However, in the first growing season of 2019, all determined values for (N, P, K) minerals content was significantly increased than the second growing season 2020.

Table (9): Effect of some biotic, abiotic resistance inducers and fungicides on the minerals, (N, P and K) content in green pods (%) of snap bean plants (cv. Paulista) during the growing seasons 2019 and 2020 under field conditions.

Treatments	Nitrogen (N) content (%) as dry weight			Phosphorus (P) content (%) as dry weight			Potassium (K) content (%) as dry weight		
	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
Bacillus subtilis	3.00	2.40	2.70	0.32	0.27	0.30	2.39	2.17	2.28
Paenibacillus polymyxa	2.73	2.50	2.62	0.36	0.28	0.32	2.44	2.26	2.35
Trichoderma harzianum	2.83	2.70	2.77	0.37	0.29	0.33	2.31	2.15	2.23
Trichoderma viride	2.53	2.30	2.42	0.34	0.29	0.32	2.36	2.17	2.27
Ascorbic acid	2.23	2.03	2.13	0.30	0.26	0.28	2.18	2.01	2.10
Bion	3.30	3.13	3.22	0.44	0.35	0.40	2.58	2.41	2.50
Potassium citrate	2.20	1.93	2.07	0.27	0.21	0.24	1.96	1.77	1.87
Salicylic acid	2.73	2.40	2.57	0.38	0.33	0.36	2.45	2.29	2.37
Domark EC 10%	3.30	2.83	3.07	0.42	0.34	0.38	2.58	2.04	2.31
Tilt EC 25%	3.13	2.23	2.68	0.31	0.24	0.28	2.28	2.08	2.18
Control	1.70	1.43	1.57	0.22	0.17	0.20	1.87	1.44	1.66
L.S.D. at 0.05									
Treatments (T)			0.301			0.013			0.208
Seasons (S)			0.128			0.005			0.089
T × S			0.426			0.018			0.295

DISCUSSION

Bean (*Phaseolus vulgaris*) is one of the valuable economic legume crops of the family Fabaceae, this important crop is considered the most important source of carbohydrates and protein for human consumption worldwide for its edible seed or green pod with global production of 26.4 million metric tons of green beans and 28.9 million metric tons of world dried bean. (Singh, 1999; FAOSTAT, 2019 and Nadeem *et al*., 2021).

Common bean rust caused by *Uromyces appendiculatus* is the major devastating disease of worldwide that causes 18 to 100% reduction of grain yield beans especially when epidemic losses occur under the high relative humidity favorable conditions (Groth and Mogen, 1978 and Alexandratos and Bruinsma, 2012). At the same time, this is especially important in the Middle East and North Africa, where moderate to large losses can occur if the disease starts early (El-Fawy and Abo-Elyousr, 2016 and Ismail and Afifi, 2019). Several investigators reported bean rust (*Uromyces appendiculatus*) as a widely spread disease in Egypt causing yield losses of dry and snap bean productions up to 70% (Abdalla, 1979; El- El-Awady and Hamed, 2015; El-Fawy and Abo-Elyousr, 2016; Ismail and Afifi, 2019; Said and Taher, 2020 and Omara *et al*. 2022).

The tested biotic and abiotic resistance inducers *Bacillus subtilis*, *Paenibacillus polymyxa*, *Trichoderma harzianum*, *Trichoderma viride*, Ascorbic acid, Bion, Potassium citrate, Salicylic acid, as well as the two tested fungicides *i.e*., Domark EC 10% and Tilt EC 25%, in *vitro*, and under field conditions in both the growing seasons (2019 and 2020) were evaluated for controlling bean rust (*U. appendiculatus* Pers.).

In this context, the tested biotic, and abiotic resistance inducers as well as two fungicides significantly inhibited germination of uredospores compared to control *in vitro*. This finding was supported by earlier studies that have demonstrated the ability of the bio-agents and chemicals of resistance inducers to inhibit the urediniospores germination rate. Mizubuti *et al*. (1995) and Zyton and Hassan (2017) tested the ability of the tested bio-agents, *B. subtilis* and *B. thuringiensis* and significantly caused reduction in the germination of *U. appendiculatus* uredospores. The obtained data also are in harmony with those reported by Ismail and Afifi (2019) and Abo-Elyousr *et al*. (2021) who indicated that uredospore germination of *U. appendiculatus in vitro* were affected by the biotic and abiotic factors, also these results agree with those obtained by Abeysinghe (2009) who mentioned that the use of rhizobacteria reduce *U. appendiculatus* uredospore germination.

Evaluation of the tested biotic and abiotic factors under field conditions for controlling bean rust, significantly reduced rust severity, AUDPC and r-value during the two successive growing seasons (2019 and 2020) compared to the control treatment. Meanwhile, all treatments significantly increased performances of bean (cv. Paulista) growth and yield parameters *i.e.*, plant height, No. of branches/plant, plant fresh

and dry weights, pod length (cm), average pod weight/plant (g) and yield (ton/fed) in the two growing seasons 2019 and 2020 as compared to untreated control.

Thus, results obtained are compatible with the findings concerning the acquire here in inducing systemic resistance (SAR) due to the treatment with the tested biotic or abiotic factors (Walters *et al.,* 2013; Prasannath, 2017 and Sarhan *et al*., 2018), Therefor, biotic and abiotic inducers have potential in agriculture regarding the managing of plant pathogens (Reddy *et al*., 2014). Bioagents treatments *i.e*., *B. subtilis*, *P. polymyxa*, *T. harzianum*, and *T. viride*, significantly reduced rust severity and (AUDPC) also the disease increase rate (r-value), the obtained data agree with several investigations that confirm the role of biological control application to suppression of plant disease and successfully inhibit the growth of the target pathogen with several disease suppression mechanisms (competition for nutrients; occlusion of pathogen; toxin inactivation; stimulation of host growth; induction of host defence;) under both greenhouse and field conditions (Elliott *et al*., 2009; O'Brien 2017 and Abo-Elyousr *et al*., 2021).

The use of bioagents treatments *i.e*., *B. subtilis*, *P. polymyxa*, *T. viride* and *T. harzianum* significantly reducing rust severity are in harmony with the several reports indicating the potential of bioagents in suppress rust disease in bean plants, (Kranz, 1981; and Mizubuti *et al*., 1995). Baker *et al.* (1985) mentioned that 3 applications/wk. of *Bacillus subtilis* in field tests reduced bean rust severity by at least 75% in 1982 and 1983 and stimulated plant growth, while *B. subtilis* treatments were more effective than the weekly mancozeb treatment. Centurion and Kimati (1994 a) and Centurion and Kimati (1994 b) reported that bacteria, *Arthrobacter* sp.; B-138, *Bacillus* sp. B-206 and *B. subtilis* AP-401 isolated from *Phaseolus vulgaris* phylloplane and soil were the best inhibitors to bean rust fungus (*Uromyces phaseoli*) as controlled *U. appendiculatus* on *Phaseolus vulgaris* by >95% and partially reduced spore viability of *U. appendiculatus*. Centurion *et al.* (1994) and Mizubuti *et al.* (1995) showed that *Bacillus* sp. isolates and *Arthrobacter* sp. reduced the number of bean rust pustules by >95%. Also inhibited germination of *U. appendiculatus* uredospores. Yuen *et al*. (2001) evaluated in the greenhouse 120 bacterial strains to control bean rust (*Uromyces appendiculatus*) found that the strains being antagonistic and effective in reducing bean rust severity.

In Egypt, Zyton and Hassan (2017) reported that *B. subtilis* and *B. thuringiensis* had greatest inhibitory effect under field conditions against pea rust (*U. pisi*) uredospores reached to 74.7 and 59.4 %. More recently, Ismail and Afifi (2018) and Abo-Elyousr *et al*. (2021) indicated that biotic factors, *Bacillus pumilus, B. subtilis, Pseudomonas putida, Trichoderma harzianum, T. asperellum* and *T. viride*, controlling bean rust disease (*Uromyces appendiculatus*) resulted in significant decrease of disease severity and AUDPC as indicated spores germination direct suppression. The treatment of chemicals inducers Ascorbic acid, Bion, Potassium citrate, Salicylic acid, were significantly reduced bean rust severity, AUDPC and r-value during the two successive growing seasons (2019 and 2020) compared to the control treatment, meanwhile were significantly effective in increasing of polyphenol oxidase (PPO), peroxidase (PO) and Catalase (CA) enzyme activities. The obtained results confirm the previous findings due to accumulation of phenolic compounds, defense-related enzymes and pathogenesis-related proteins and inducing the systemic acquired resistance that following chemicals and biotic inducers treatment in plants have potential in plant diseases management (Sticher *et al.,* 1997; Walters *et al.,* 2013 and Reddy *et al.,* 2014). These results are in good agreement with several studies which have showed that accumulation of phenolics and defense-related enzymes can cause defense in plants against diseases attack (Prasannath, 2017 and Sarhan *et al*., 2018). Görlach *et al.* (1996); Bovie *et al.* (2004) and Iriti *et al.* (2004) reported that Bion (BTH) induces systemic acquired resistance by increasing the pathogenesis-related proteins (PR), activity of oxidative enzyme and accumulation of phenolic compounds.

Iriti and Faoro, (2003) showed that in bean cultivars against rust (*Uromyces appendiculatus*) Bion induce resistance and controlled the bean rust over all the tested cultivars. The same was obtained by Guo and Stotz, 2007; Azami-Sardooei *et al.* (2013) and Bán *et al.* (2017) who indicated that foliar applications of Bion reduced *B. cinerea* on cucumber and sunflower and white mold (*S. sclerotiorum*) disease on bean besides both systemic and localized inductions of resistance were observed. Gaffney *et al.* (1993); Sticher *et al.* (1997) and Kumar, (2014) reported that Salicylic acid (SA) plays an important role in the induction of plant defense as plant hormone against plant diseases via various host-pathogen

interactions. Reglinski, *et al.* (1997) and Reglinski *et al.* (2001) found that Salicylic acid caused a reduction to *S. sclerotiorum* and was effective elicitor and induced phenylalanine ammonia-lyase (PAL) enzyme activity48h post-inoculation. Abada *et al*. (2009) reported that spraying pea plants with some inducing resistance chemicals *i.e.,* Bion, potassium monobasic phosphate, zinc sulphate and salicylic acid significantly reduced the severity of powdery mildew of pea, also they increased number of green pods/plant, weight of green pods/plant and plant height, compared with control treatment under field and greenhouse conditions.

Recently Abdel-Kader *et al*., 2011; Idrees *et al.*, 2011; Wang *et al.,* 2012; Ahmed, 2016 and Kouzai *et al*., (2018) reported that salicylic acid, potassium sorbate, benzoic and sorbic acids and their salts sodium benzoate improved the defense system via enhancing the activities of various antioxidant enzymes such as, phenylalanine ammonia-lyase (PAL), peroxidase (PO), polyphenoloxidase (PPO), and etc., against *S. sclerotiorum* and *Rhizoctonia solani* with a significant increase in the roots and shoots fresh and dry weight compared with control treatment demonstrated that salicylic acid (SA) and benzothiadiazole (BTH), because of inducing plant defense responses has been widely used to protect crops from diseases. Whereas Sarhan *et al*. (2018) demonstrated that salicylic acid (SA) and Bion [benzothiadiazole, (BTH)], as chemical inducers significantly reduced bean white mold disease, and highly increased crop parameters and vegetative. El-Fawy and Abo-Elyousr (2016) and Ismail and Afifi (2018) found that under greenhouse and field conditions, application of the chemical inducer phosphoric acid, pyrocatechol, fulvic and ascorbic, benzoic acid and Bion, followed by salicylic acids significantly decreased bean rust (*Uromyces appendiculatus*) severity and AUDPC and (r-value) in addition to significantly increase of common bean growth parameters, total carbohydrates, protein and photosynthetic pigments in bean plants over fungicide treatment and untreated control, meanwhile enhanced the activities of oxidative enzymes in bean plants.

CONCLUSIONS

The present study indicated that spraying biotic and abiotic resistance inducers *i.e., Bacillus subtilis*, *Paenibacillus polymyxa*, *Trichoderma harzianum*, *Trichoderma viride*, ascorbic acid, Bion, potassium citrate, salicylic acid, as well as the two tested fungicides *i.e*., Domark EC 10% and Tilt EC 25% could play a significant role against snap bean rust (*Uromyces appendiculatus*) as reduced the disease incidence and severity in *vitro* and under field conditions, mainly through eliciting the systemic resistance via increasing the activities of the peroxidase, polyphenol oxidase, and catalase antioxidant enzymes, and phenolic contents. Besides, promote plant growth and increases the marketable yield and improves the snap bean green pods chemical composition. Our investigation provides better understanding for such biotic and abiotic inducers on the field scale as provide an eco-friendly practical management of snap bean rust can be introduced to integrated disease management.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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