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RESEARCH

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Phytopathological and biochemical impacts of *Trichoderma harzianum* and certain plant resistance inducers on faba bean root rot disease

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Abstract

Background Faba bean attacked by soil-borne pathogens causing root rot disease. This disease has serious damage to both plant stand and produced yield. The present study aimed to evaluate effectiveness of the bioagents; *Trichoderma harzianum* and some plant resistance inducers as fungicide alternatives against root rot disease incidence at both pre- and post-emergence growth stages.

Results Under open greenhouse conditions**,** the incidence of faba bean root rot in pre- and post-emergence growth phases was considerably reduced by using six inorganic salts and five antioxidants individually or combining with each other or with the bio-stimulator *T. harzianum* that exceeded the used fungicide, Rhizolex-T. Application of enervit agitated the highest significant defensive impact during pre-emergence stage versus root rot incidence (5.0%), followed by calcium sulfate and [cysteine + *T. harzianum*] (6.7%). At post-emergence stage, majority of the treatments completely suppressed (100.0%) root rot incidence, except vitamax plus and the fungicide (Rizolex-T) which expressed by 91.7 and 18.8%, respectively. Duplicate irrigations of 23 treatments after faba bean dressing improved the synthesis of different protein contents with the 2nd of which enhanced higher protein contents than the 1st one, except [*T. harzianum* + vitamin E + vitamin C + enervit + selenium + vitamax plus], [*T. harzianum* + vitamax plus] and cysteine. Disodium phosphate induced the highest catalase (CAT) activity (1820.8 and 1677.2 U/g FWt) after both irrigations. [*T. harzianum* + vitamax plus] and vitamin E induced the highest peroxidase (POD) activity 217.4 and 356.9 U/g FWt after 1st and 2nd irrigations, respectively. Disodium phosphate and [*T. harzianum* + vitamin E + vitamin C + enervit + selenium + vitamax plus] induced the highest chitinase (CHIA) activity 52.8 and 54.4 U/g FWt after 1st and 2nd irrigations, respectively. Application of disodium phosphate, calcium sulfate, potassium metabisulfite, sodium sulfate, cysteine, [cysteine + potash alum], enervit, vitamin E, [vitamin E + vitamin C + enervit + selenium + vitamax plus], [*T. harzianum* + enervit], [*T. harzianum* + selenium], [*T. harzianum* + vitamin E], [*T. harzianum* + vitamin E + vitamin C + enervit + selenium + vitamax plus] and vitamin C stimulated the formation of new protein bands on SDS-PAGE after the 2nd irrigation treatment.

Conclusions Such treatments are considered good and environmentally safe alternatives against root diseases for getting rid of the negative effects of fungicides.

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Keywords Antioxidants, Biochemical studies, Faba bean, Inorganic salts, Root rot, *Trichoderma harzianum*

Background

The root diseases cause acute deterioration to most agrarian crops through the various growth stages of plants leading to large losses of yield and quality. *Vicia faba* L. (faba bean) is an essential food and feed legume crop in the world, especially developing countries. Seeds of faba bean plant are rich in protein content, mineral nutrients and some bioactive compounds (Etemadi et al. 2019). The attack of diseases induced by soil-borne pathogens on cultivated crops is considered the major factor of plant stands and crop damages in various countries. Reason for the occurrence of the destructive root rot disease in faba bean plant is the fungi *Sclerotium rolfsii*, *Fusarium solani* and *Rhizoctonia solani* (Long et al. 2022). The disease of root rot takes place on the crops growing season from up growth over surface of the soil to seedling phase. It also can infect seeds leading to pre-emergence infection which necessitates replanting the missed hills or dead plants (El-Mougy et al. 2017). Due to the economic significance of faba bean crop in Egypt, it is produced for centuries and consecutively cultivated on the same lands which lead to high build-up of pathogen inocula and serious yield damages.

In spite of the intensive uses of fungicides that chemically synthesized, they don't present satisfying control for root diseases. Lately, there is an increase in utilizing alternatives for chemical fungicides to obviate their negative impacts on environment and human health (Neupane and Baysal-Gurel 2022). Fungicide alternatives can be considered as one possible means of controlling plant pathogens without causing any harm to host plants (Megahed et al. 2013a; El-Mougy et al. 2017). Various procedures were utilized for research of the biologically energetic substances such as the systematic investigation of the interaction between plant products and microorganisms. This procedure is considered as an important source of beneficial factors for controlling the survival of microbes in diverse application domains (Megahed et al. 2013b). The vegetarian products with antimicrobial characteristics could be used in food preservation processes as major antimicrobial components or as an adjuvant for improving the function of other antimicrobial agents (Kaur and Arora 1999). Some interesting alternatives to fungicides application include the use of some inorganic salts or some antioxidants, which are known with their antimicrobial characteristics and protecting to environment and human to control some plant diseases (Turkkan 2015).

Plant defense-concerned enzymes like CAT, POD and CHIA perform a pivotal part in the host resistance of plants (Xie et al. 2017) and could be considered as a part of plant defense against pathogens (Han et al. 2016). Catalases (CATs) were the first discovered antioxidant enzymes that catalyze the conversion of H_2O_2 into H_2O and O_2 in the cells suffering from environmental stress. They play a significant part in plants defense and aging and protecting them from oxidative damage. CATs are situated in all major H_2O_2 producing sites of the cell (mitochondria, peroxisomes, cytosol and chloroplast) in plants. Multiple molecular forms of CAT isoenzymes elucidate their multilateral role within the plant system. The modulation of H_2O_2 by the CAT isoenzymes within specific tissues at certain periods and growing stages overlapped with the signal transduction process in plants (Palma et al. 2020). Peroxidases (PODs) are hemoproteins with a wide structural variability that catalyze the redox reaction between H_2O_2 and some redactors. In plants, PODs are implicated in many cellular activities like auxin metabolism, cell wall modifications, defense against pathogens, aging, tolerating salts and heavy metals, and stress responses. These enzymes can be considered as biomarkers indicating biotic or abiotic stresses (Begovic et al. 2017). Chitinases (CHIAs) exist in diverse microorganisms including fungi, bacteria, plants and insects. When plant cells are exposed to pathogens, CHIAs are strongly expressed and play an important role versus fungal pathogens. Plant CHIAs are implicated in many abiotic stress responses such as wounding, cold, osmotic pressure, heavy metals stress and salinity (Vaghela et al. 2022).

The aim of this study is to evaluate the impacts of the bio-stimulator, *T. harzianum,* inorganic salts and some antioxidants individually and in combination with each other versus faba bean root rot pathogens under open green-house conditions. Estimation of the disease incidence and determination of the protein content and the specific activities of the potential biochemical indicators; catalase, peroxidase and chitinase was carried out.

Methods

Materials

Vicia faba L. *cv.* seeds (Giza 3) were brought from Vegetable Crops Research Dept., Agricultural Research Centre, Giza, Egypt. Disodium phosphate, calcium sulphate, potassium metabisulphite, sodium sulphate, cysteine, potassium aluminium sulphate (potash alum), vitamin C, vitamin E, selenium, vitamax plus and enervit were

bought from Al-Gamhoria Co. Ltd. for chemicals and medicinal instruments, Cairo, Egypt. Phenyl methyl sulphonyl fluoride (PMSF), bovine serum albumin (BSA), guaiacol, chitin, dinitrosalicylic acid (DNS) and N-acetyl glucosamine (NAGA) were bought from Sigma Chemical Co. All of other chemicals were of research category. The bioagent *T. harzianum* was acquired from cultural collection unit, Plant Pathology Dept., NRC, Egypt.

Experimental design

The inorganic salts (disodium phosphate, calcium sulphate, potassium metabisulfite, sodium sulphate, cysteine and potash alum) and the antioxidants (vitamin C, vitamin E, selenium, enervit and vitamax plus) were dissolved in sterilized distilled water in 2% concentrations (w:v) after El-Mougy and Abdel-Kader (2018). The biostimulator *T. harzianum* was sub-cultured in PD broth medium and incubated for 7 days at 28 °C, then the conidial media suspension was adjusted at 10^8 spores/ml using haemocytometer slide. The fungicide Rizolex-T (50WP) was utilized as a comparable treatment at concentration of 3 g/Kg seeds for the seed dressing and 1 g/1 l for the next both irrigations.

The faba bean seeds of *cv.* Giza 3 were soaked for 60 min in 100 ml of each inducer that used for seed dressing treatment and directly cultivated in washed, air-dried and sterilized plastic pots (40 cm diameter) loaded with 5 kg/pot loamy-sand soil that taken from a field well recognized by the authors throughout preceding searches as naturally heavily infested with soil-borne faba bean root rot pathogens; *Fusarium solani, Sclerotium rolfsii* and *Rhizoctonia solani* (El-Mougy et al. 2017) [6 seeds/pot with 10 replicates for each inducer treatment]. Treatments consist of 23 inducers, Rizolex-T (50WP) treated control and untreated control (Table 1) in a complete randomized design. All pots were conducted under open greenhouse conditions, Plant Pathology Dept., NRC, Egypt during growing season 2021–22. All treated and untreated pots were daily irrigated with suitable enough tab water only. Irrigation with all inducer treatments

Table 1 The influence of *Trichoderma harzianum,* salts and oxidants on faba bean root rot disease incidence

Treatment (2% w:v)	Root rot incidence %			
	Pre-emergence	Reduction*	Post-emergence	Reduction*
Disodium phosphate	8.3e	78.3e	0.0c	100.0c
Calcium sulfate	6.7e	82.5e	0.0c	100.0c
Potassium metabisulfite	11.7d	69.5d	0.0c	100.0c
Sodium sulfate	11.7d	69.5d	0.0c	100.0c
Cysteine	21.7b	43.3b	0.0c	100.0c
Potash alum	13.3 cd	65.3 cd	0.0c	100.0c
Cysteine + potash alum	8.3e	78.3e	0.0c	100.0c
Cysteine + *Trichoderma harzianum*	6.7e	82.5e	0.0c	100.0c
Potash alum + *T. harzianum*	13.3 cd	65.3 cd	0.0c	100.0c
Cysteine + potash alum + *T. harzianum*	8.3e	78.3e	0.0c	100.0c
Enervit	5.0e	86.9e	0.0c	100.0c
Selenium	11.7d	69.5d	0.0c	100.0c
Vitamin E	11.7d	69.5d	0.0c	100.0c
Vitamin C	13.3 cd	65.3 cd	0.0c	100.0c
Vitamax plus	23.3b	39.2b	2.2b	91.7b
Trichoderma harzianum + enervit	16.7c	56.4c	0.0c	100.0c
T. harzianum + selenium	20.0b	47.8b	0.0c	100.0c
T. harzianum + vitamin E	8.3e	78.3e	0.0c	100.0c
T. harzianum + vitamin C	13.3 cd	65.3 cd	0.0c	100.0c
T. harzianum + vitamax plus	16.7c	56.4c	0.0c	100.0c
Vitamin E + vitamin C + enervit + selenium + vitamax plus	15.0c	60.8c	0.0c	100.0c
T. harzianum + vitamin E + vitamin C + enervit + selenium + vitamax plus	13.3 cd	65.3 cd	0.0c	100.0c
Trichoderma harzianum (10^8 spores/ml)	20.0b	47.8b	0.0c	100.0c
Rizolex-T (50WP) (1 gm/l)	35.0a	8.6a	21.6a	18.8a
Untreated control	38.3a	–	26.6a	–

Mean values within columns followed by the same letter are not significantly different at $P \leq 0.05$

$$\text{Reduction \%} = \frac{\text{Untreated control} - \text{treatment}}{\text{Untreated control}} \times 100$$

was used twice as additional support for seed dressing application after 1 and 3 weeks from seed cultivation at the rate of 1 l/pot each time. The first irrigation was after one week from cultivation, and the second was 2 weeks later from the first one. In both irrigations, each pot was re-treated with each inducer except untreated control irrigated with tab water only. The faba bean leaves were collected 2 weeks after inducers application in both irrigation times (3 and 5 weeks from seed sown time) for protein and enzymes determination. Root rot disease incidence percent was recorded as pre- and post-emergence after 15 and 45 days from sowing date referring to the number of sowing seeds.

Extraction of total proteins

Proteins were extracted according to (Lanna et al. 1996; Megahed et al. 2019). One-gram fresh weight (FWt) was ground in a mortar containing liquid nitrogen and the resulting powder was mashed for 30 s in 3 ml extraction buffer [0.05 M Na-phosphate buffer pH 6.5 containing 1 mM PMSF] and centrifuged at 20,000 x*g* for 30 min. at 4 °C to obtain the supernatant which was protected at − 20 °C for next determinations.

Protein determination

Protein contents were determined by the dye binding assay method using BSA as a standard (Bradford 1976) utilizing Shimadzu UV-2401 spectrophotometer.

Enzymes assays

CAT activity assay

CAT was assayed in 3 ml 0.05 M K-phosphate buffer pH 7.0 comprising 0.02 M H_2O_2 and the enzyme sample. The H_2O_2 decomposition was followed up as a reduction in absorbance at 240 nm for 3 min. One CAT unit was determined by calculating the consumption of 1 µmol H_2O_2 per min at 25 °C taking into consideration 43.6 M^{-1} cm^{-1} extension coefficient of H_2O_2 (Aebi 1984).

POD activity assay

POD was assayed by estimating the absorbance change at 470 nm of guaiacol oxidation in existence of H_2O_2 and the enzyme sample every 30 s. intervals. One POD unit was known as the amount of enzyme yielding a change of 1 O.D. min^{-1} (Johri et al. 2005).

CHIA activity assay

CHIA was assayed using colloidal chitin (substrate) and DNS for estimating the reducing sugars. Colloidal chitin was prepared by milting 25 gm. chitin powder, then suspending in 250 ml 85% H_2SO_4 and hold at 4 °C for 24 h. Blending this mixture in 2 l dH_2O, then centrifuging (2500 x*g*, 20 min), repeating this step twice and adjusting the colloidal suspension to pH 7.0 in the final wash and collecting the colloidal chitin via centrifugation and keeping at 4 °C. For CHIA assay, a mixture of l ml 1% colloidal chitin in 50 mM Na-acetate buffer pH 6.6 and 1 ml sample was incubated for 1 h. at 37 °C and then stopping this reaction by 1 ml DNS [0.25 M NaOH, 0.04 M DNS, 0.02 M phenol, 4 mM sodium sulfite and 0.7 M sodium potassium tartrate]. Incubation of this mixture at 100 °C for 10 min. to develop the color, centrifuge for 10 min at 7500 x*g*, and then measuring the supernatant at 540 nm. A calibration curve was plotted using NAGA. One CHIA unit represents the enzyme amount that released 1 µmol NAGA min^{-1} (Ried and Ogrydziak 1981; Boller and Mauch 1988).

Electrophoretic analysis on SDS-PAGE

The extracted proteins of different treatments were analyzed on 12% polyacrylamide SDS-PAGE (Weber and Osborn 1969; Laemmli 1970) and Coomassie brilliant blue R-250 was utilized for staining. The gels were analyzed using Syngene™ Ingenius 3 Gel Documentation System software.

Statistical analysis

General Linear Model option of the Analysis System SAS (SAS, 1996) was utilized to accomplish the variance analysis. Duncan's Multiple Range Test at $p \leq 0.05$ levels was utilized for means separation (Bailey 1997).

Results

Disease incidence

Data presented in Table 1 reveal the percentage of faba bean root rot disease incidence at pre- and post-emergence stages. The average disease incidence of the applicable seed dressing treatments supported by the 1st irrigation significantly reduced the root rot incidence at pre-emergence stage ranging from 5.0 to 23.3% compared to 35.0% for Rizolex-T (50WP) treatment and 38.3% for untreated control. The highest significant protective inducers against root rot pathogens invasion were enervit (5.0% incidence) then calcium sulphate and [cysteine + *T. harzianum*] (6.7% incidence), followed by [cysteine + potash alum] (8.3% incidence). On the other hand, the lowest protective inducers were vitamax plus (23.3% incidence) then cysteine (21.7% incidence), followed by [*T. harzianum* + selenium] and *T. harzianum* (20.0% incidence). At post-emergence phase, an interesting extending effect was monitored for all applied treatments that supported by 2nd irrigation and completely suppressed 100.0% root rot incidence, except vitamax plus and the fungicide (Rizolex-T) (50WP) which expressed by 91.7 and 18.8%.

Protein content

Protein contents were determined in faba bean plants treated with individual and mixed combinations of inorganic salts, antioxidants and *T. harzianum* bio-inducer related to BSA as standard protein (Table 2). The employed additional two irrigations of these inducers caused either a decrease or increase in the entire protein content in treated plants in both irrigation times. Selenium and cysteine induced the highest protein contents (0.479 and 0.467 mg/g FWt), while [*T. harzianum* + vitamin E + vitamin C + enervit + selenium + vitamax plus] and disodium phosphate induced the lowest protein contents (0.187 and 0.200 mg/g FWt) after the 1st irrigation. Potassium metabisulfite and [cysteine + potash alum + *T. harzianum*] induced the highest protein contents (0.636 and 0.600 mg/g FWt), while [*T. harzianum* + vitamin E + vitamin C + enervit + selenium + vitamax plus] and [*T. harzianum* + vitamax plus] induced the lowest protein contents (0.176 and 0.232 mg/g FWt) due to the 2nd irrigation treatment. All treatments after the 2nd irrigation treatment induced higher protein contents than the 1st one, except the treatments [*T. harzianum* + vitamin E + vitamin C + enervit + selenium + vitamax plus], [*T. harzianum* + vitamax plus] and cysteine. Different protein contents were elicited after the two irrigations except [cysteine + *T. harzianum*], [potash alum + *T. harzianum*] and enervit had the same protein content (0.575 mg/g FWt) after the 2nd one (Fig. 1) compared to untreated controls.

Enzymes activity

Catalase, peroxidase and chitinase activities were increased in some treated faba bean plants after both irrigations. There were some treatments induced higher

Table 2 Effect of *Trichoderma harzianum*, salts and antioxidants on protein contents and enzymes activity of faba bean against root rot infection

Treatment (2% w:v)	Total protein mg/g FWt		CAT specific activity (U/g FWt)		POD specific activity (U/g FWt)		CHIA specific activity (U/g FWt)	
	1st appl	2nd appl	1st appl	2nd appl	1st appl	2nd appl	1st appl	2nd appl
Disodium phosphate	0.200	0.316	1820.8	1677.2	104.6	62.7	52.8	38.6
Calcium sulfate	0.280	0.537	783.0	455.3	212.3	116.7	35.6	24.0
Potassium metabisulfite	0.322	0.636	428.8	72.3	117.7	49.3	27.4	23.5
Sodium sulfate	0.256	0.596	94.2	573.3	124.2	59.3	35.6	26.3
Cysteine	0.467	0.448	346.0	529.9	83.0	118.3	30.8	27.1
Potash alum	0.342	0.446	272.7	416.6	128.9	124.7	28.5	22.7
Cystiene + potash alum	0.322	0.400	1172.1	170.5	184.6	60.1	40.0	28.2
Cystiene + *Trichoderma harzianum*	0.228	0.575	833.3	463.5	148.2	110.9	34.3	25.1
Potash alum + *T. harzianum*	0.258	0.575	82.3	1570.4	119.3	106.1	33.2	26.7
Cystiene + potash alum + *T. harzianum*	0.366	0.600	330.2	412.9	39.0	85.1	24.9	26.9
Enervit	0.296	0.575	650.8	702.0	120.6	72.8	32.6	29.9
Selenium	0.479	0.528	126.7	205.2	165.5	45.0	25.5	27.9
Vitamin E	0.386	0.508	249.6	77.3	108.6	356.9	26.1	29.3
Vitamin C	0.387	0.537	293.3	642.8	202.0	83.5	28.7	25.0
Vitamax plus	0.369	0.460	119.2	979.6	109.6	129.1	30.0	28.3
T. harzianum + enervit	0.282	0.501	239.8	244.6	88.5	59.3	26.7	25.8
T. harzianum + selenium	0.320	0.462	573.4	597.0	167.7	97.8	28.9	29.0
T. harzianum + vitamin E	0.385	0.390	343.9	501.3	94.0	40.6	28.9	27.9
T. harzianum + vitamin C	0.231	0.434	162.9	491.4	199.1	96.8	36.3	26.0
T. harzianum + vitamax plus	0.428	0.232	750.0	207.8	217.4	153.0	34.7	33.8
Vitamin E + vitamin C + enervit + selenium + vitamax plus	0.330	0.384	417.2	484.4	124.2	106.1	32.4	28.9
T. harzianum + vitamin E + vitamin C + enervit + selenium + vitamax plus	0.187	0.176	797.4	1408.0	202.0	267.2	38.7	54.4
T. harzianum (10^8 spores/ml)	0.316	0.411	177.0	446.6	135.1	61.0	26.3	25.1
Rizolex-T (50WP) (1 gm/l)	0.350	0.656	990.6	523.0	337.2	70.7	40.0	27.3
Untreated control	0.472	0.332	518.7	257.0	300.8	45.0	28.8	30.2

*The specific activity is expressed as unit /gram fresh weight (U/g FWt)

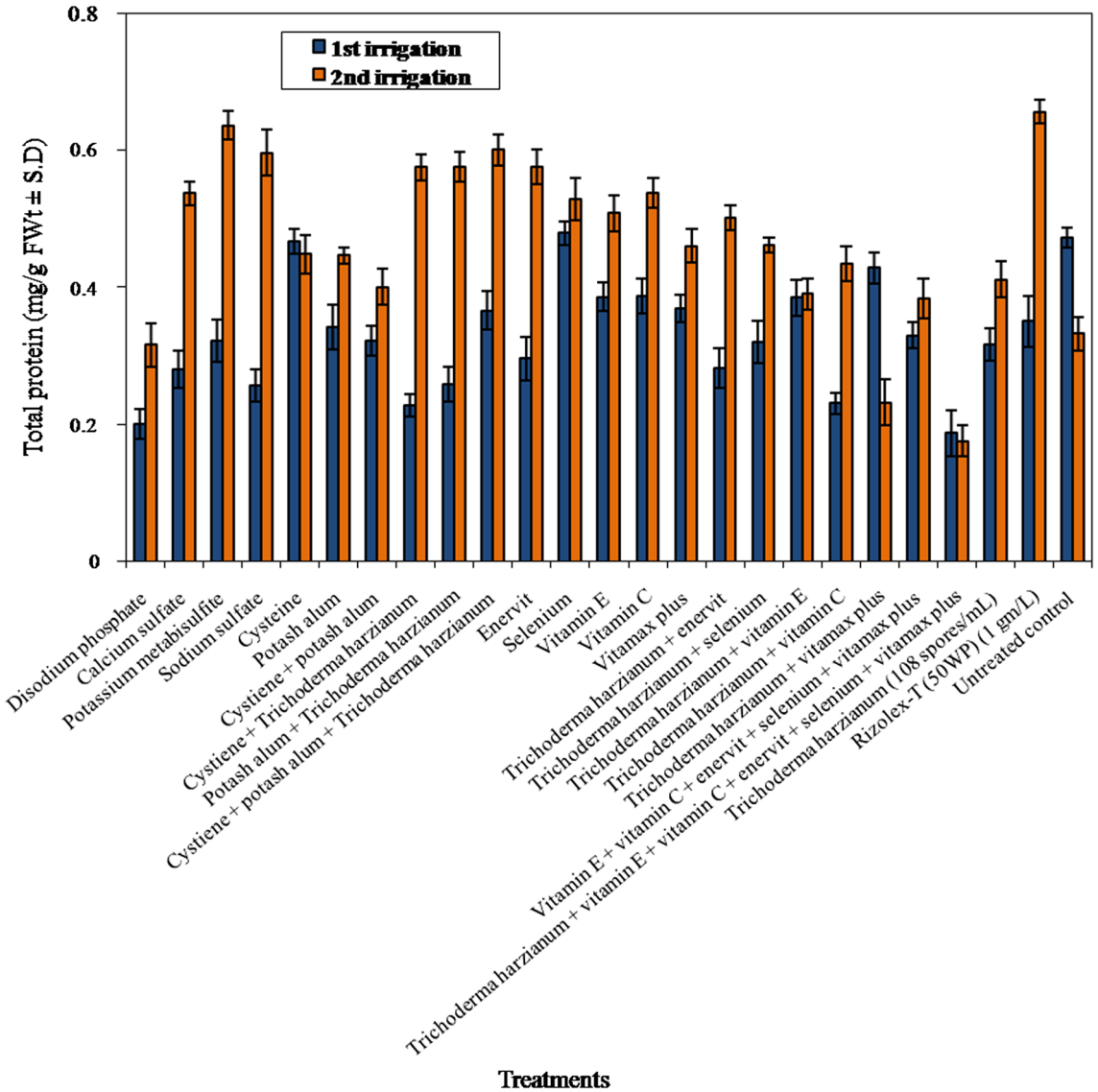

Fig. 1 Effect of seed dressing followed by two irrigations with *Trichoderma harzianum*, salts and antioxidants individually or in combination on protein contents of faba bean plants against root rot infection

CAT, POD and CHIA specific activities, after the 2nd irrigation than the 1st one, while others induced lower activities. Both irrigation times elicited different CAT, POD and CHIA specific activities (Table 2). Disodium phosphate induced the highest CAT specific activity (1820.8 U/g FWt), while [potash alum + *T. harzianum*] induced the lowest CAT specific activity (82.3 U/g FWt) after the 1st irrigation. Disodium phosphate also induced the highest CAT specific activity (1677.2 U/g FWt), while potassium metabisulfite induced the lowest CAT specific activity (72.3 U/g FWt) after the 2nd irrigation time (Fig. 2) than the untreated control.

[T. harzianum + vitamax plus] induced the highest POD specific activity (217.4 U/g FWt), while [cysteine + potash alum + *T. h*arzianum] induced the lowest POD specific activity (39.0 U/g FWt) after 1st irrigation. Vitamin E induced the highest POD specific activity (356.9 U/g FWt), while [*T. harzianum* + vitamin E] induced the lowest POD specific activity (40.6 U/g FWt) after the 2nd irrigation treatment (Fig. 3) than the untreated control. Disodium phosphate induced the highest CHIA specific activity (52.8 U/g FWt), while [cysteine + potash alum + *T. harzianum*] induced the lowest CHIA specific activity (24.9 U/g FWt) after the 1st irrigation. The treatment

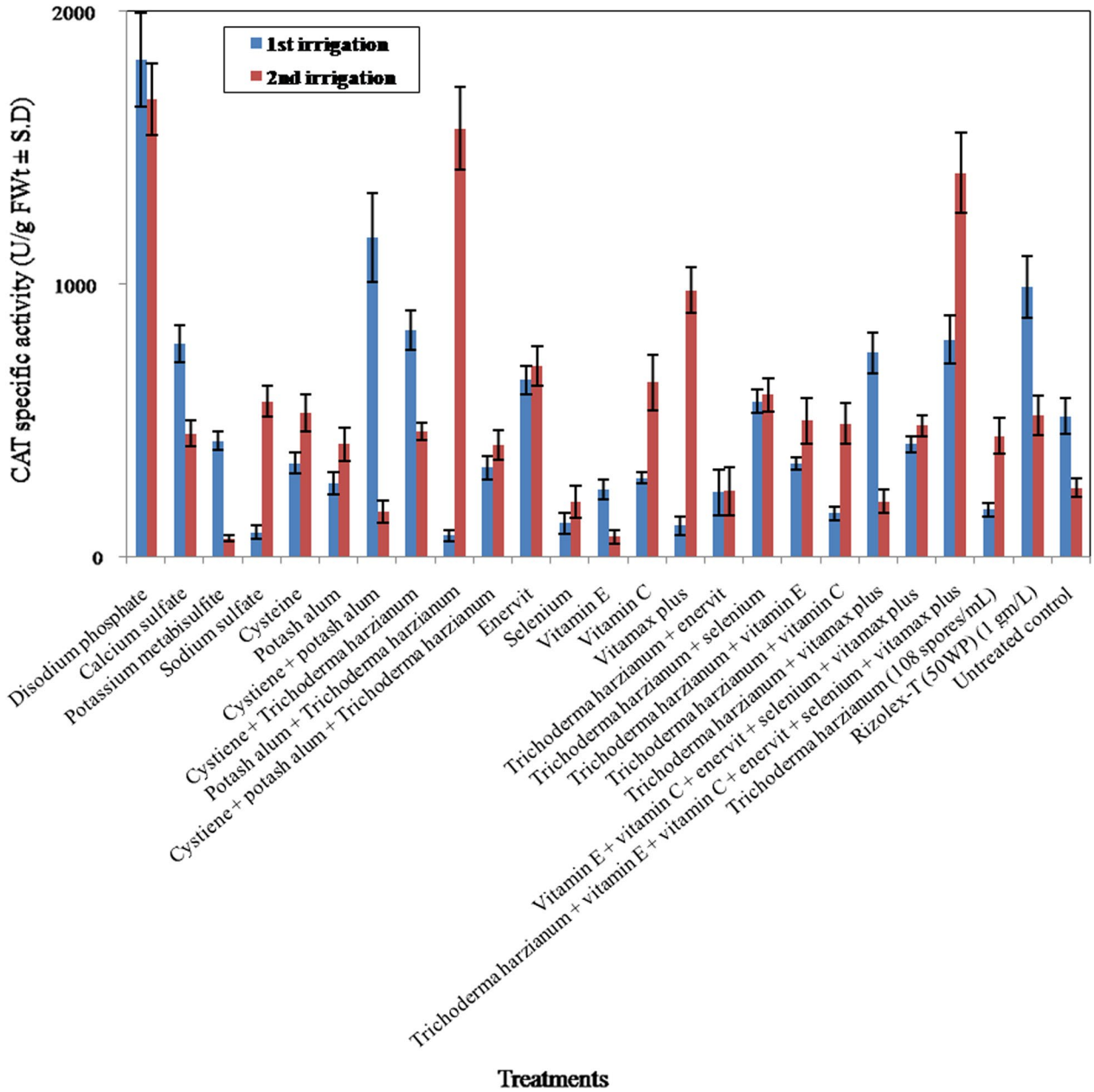

Fig. 2 Effect of seed dressing followed by two irrigations with *Trichoderma harzianum*, salts and antioxidants individually or in combination on CAT specific activity of faba bean plants against root rot infection

[*T. harzianum* + vitamin E + vitamin C + enervit + selenium + vitamax plus] induced the highest CHIA specific activity (54.4 U/g FWt), while potash alum induced the lowest CHIA specific activity (22.7 U/g FWt) after the 2nd irrigation time (Fig. 4) compared to untreated control.

Electrophoretic analyses on SDS-PAGE

The protein patterns (75 µg protein definite amount) of the crude extracts for all faba bean plant treatments were compared by analysis on 12% SDS-PAGE (Fig. 5). Data analysis of SDS-PAGE protein patterns (Table 3) of different treated faba bean plants with each inducer showed that, the highest number of protein bands

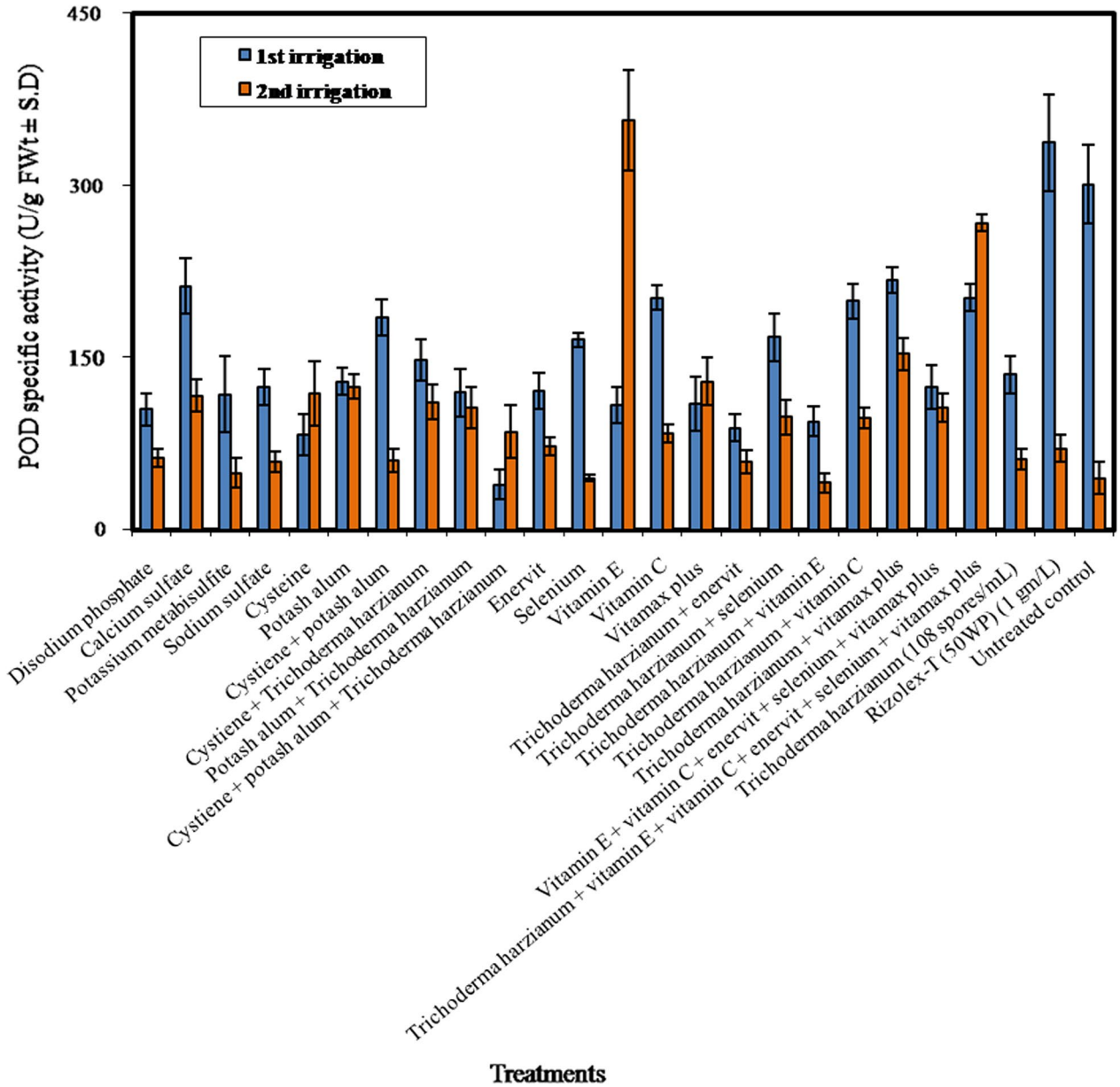

Fig. 3 Effect of seed dressing followed by two irrigations with *Trichoderma harzianum*, salts and antioxidants individually or in combination on POD specific activity of faba bean plants against root rot infection

was formed after the 2nd irrigation time of [*T. harzianum* + enervit] (11 protein bands). The lowest number of protein bands was recorded at different treatments after both irrigation times (5 protein bands). Potash alum, [cysteine + *T. harzianum*], [potash alum + *T. harzianum*], [cysteine + potash alum + *T. harzianum*], selenium, vitamax plus and [*T. harzianum* + vitamin C] irrigation treatments synthesized the same number of protein bands after both applications.

Discussion

Many studies have been performed to develop modern and eco-friendly methods to alternate the use of chemical fungicides for plant diseases control. The fungus, *Trichoderma* utilization was recorded to be the most promising and efficient bio-control agent. This antagonistic genus is controlling wide range of microbes and its mode of action includes hyper-parasitism, nutrient competition, cell wall degrading enzymes and antibiosis

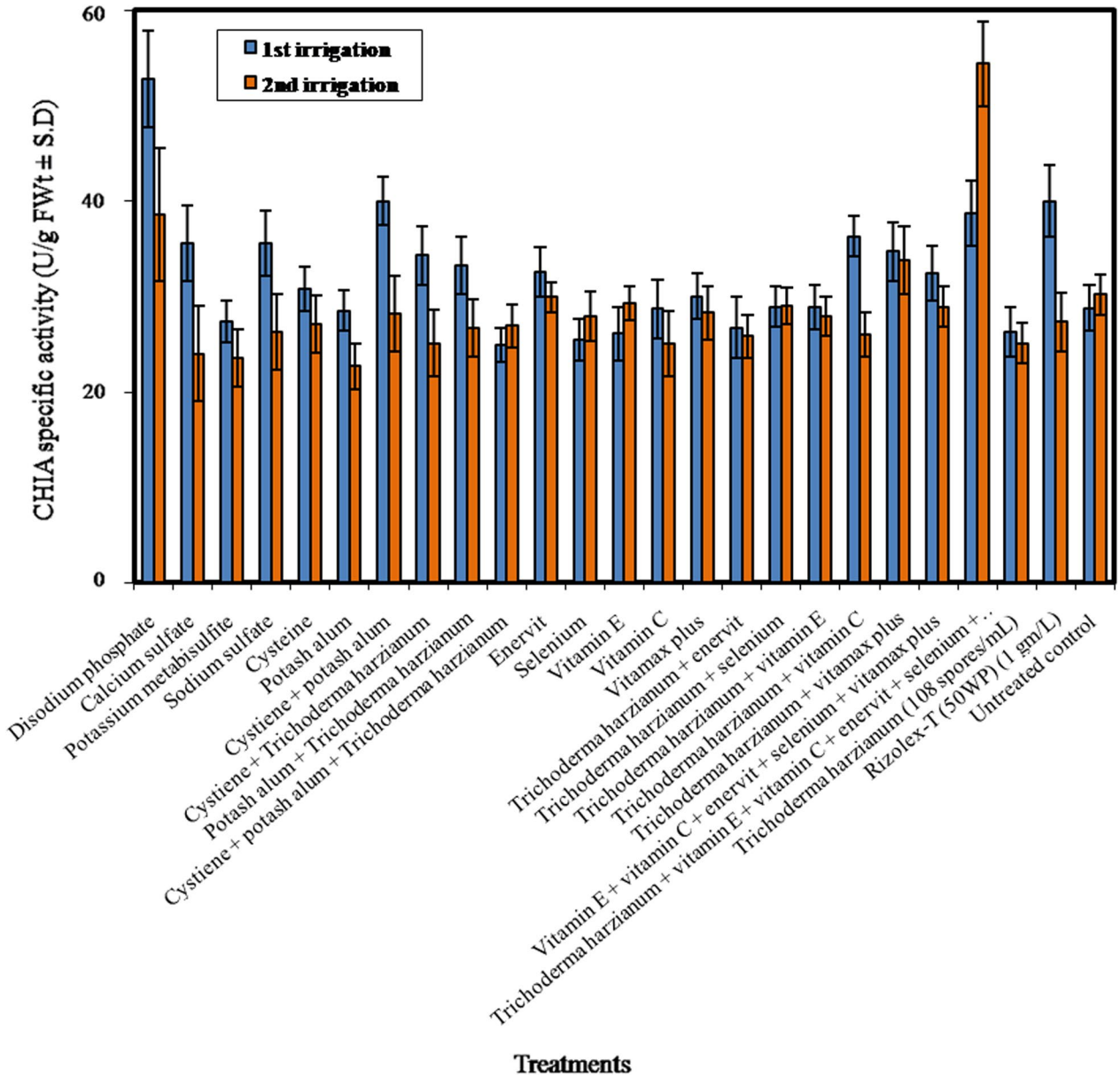

Fig. 4 Effect of seed dressing followed two irrigations with *Trichoderma harzianum*, salts and antioxidants individually or in combination on CHIA specific activity of faba bean plants against root rot infection

(Chet et al. 1997). Furthermore, many researchers followed up the procedure of utilizing organic and inorganic salts for their efficiency against plant diseases. They attributed their effectiveness to the phenomena showing that the applied salts may ultimately be used for the control of plant diseases. Consistently to this conclusion, the present study showed that potassium metabisulfite and potash alum could reduce root rot disease incidence. Concerning Alum antimicrobial effect, Bnyan et al. (2014) stated that the antimicrobial activity of potash alum against the growth of several microbes in vitro. Also, potassium and sodium salts have been registered to have antifungal proprieties. The control of some phytopathogenic fungi by utilizing mono K-phosphate and di K-phosphate was evaluated as potential alternatives to synthetic fungicides (Umit 2015). Also, sodium benzoate and sodium metabisulfite revealed a strong complete inhibition of mycelial growth and spore germination of several species of the genus *Fusarium* (Mecteau et al. 2008).

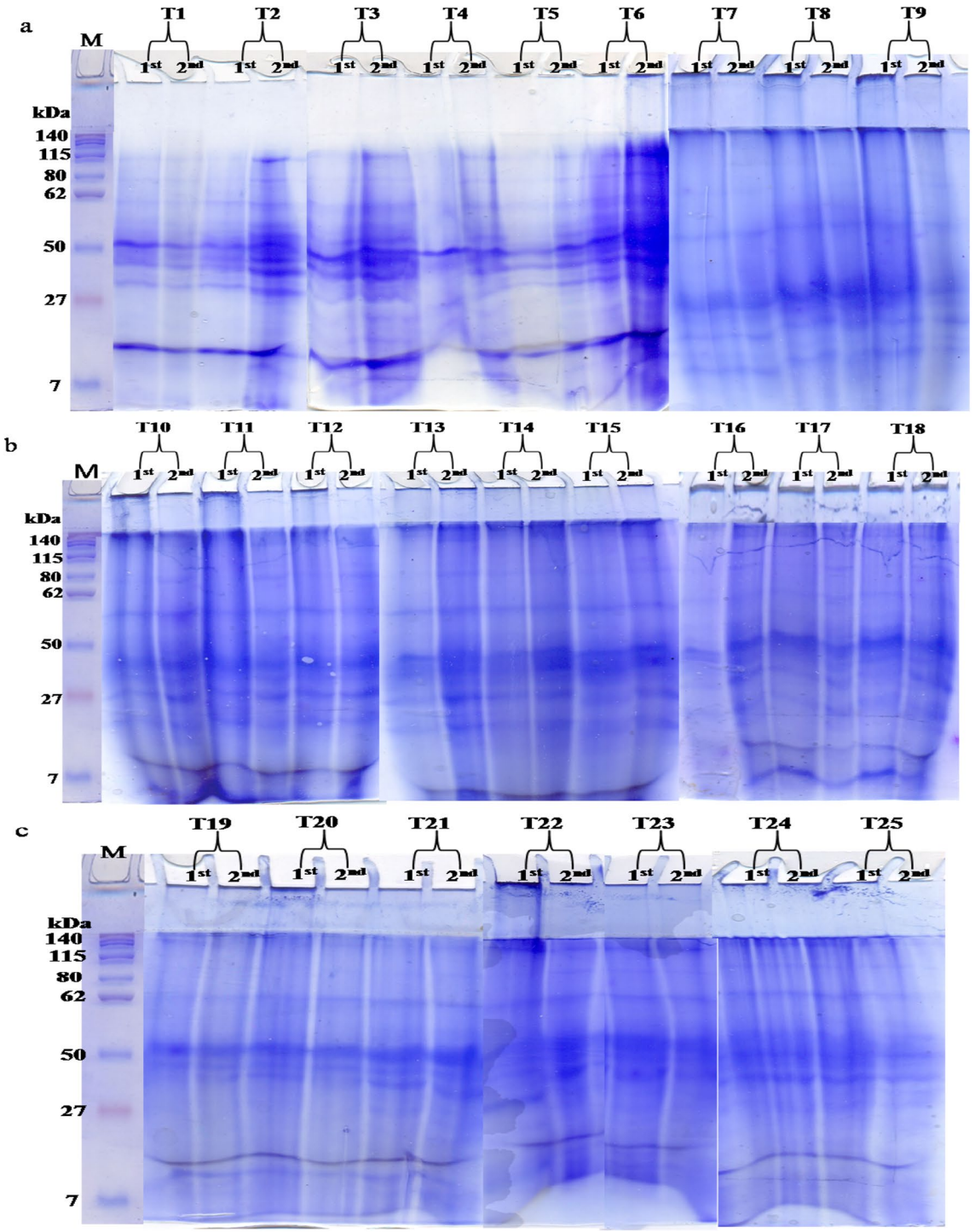

Fig. 5 12% SDS-PAGE showing the effect of different inducers after both irrigation times on protein pattern of faba bean plants against root rot infection; (M) standard protein markers, **a** (T1) disodium phosphate, (T2) calcium sulfate, (T3) potassium metabisulfite, (T4) sodium sulfate, (T5) cysteine, (T6) [cysteine + potash alum], (T7) potash alum, (T8) [cysteine + *Trichoderma harzianum*] and (T9) [potash alum + *T. harzianum*]. **b** (T10) [cysteine + potash alum + *T. harzianum*], (T11) Enervit, (T12) Selenium, (T13) Vitamin E, (T14) vitamax plus, (T15) [Vitamin E + vitamin C + enervit + selenium + vitamax plus], (T16) [*T. harzianum* + enervit], (T17) [*T. harzianum* + selenium] and (T18) [*T. harzianum* + vitamin E]. **c** (T19) [*T. harzianum* + vitamax plus], (T20) [*T. harzianum* + vitamin E + vitamin C + enervit + selenium + vitamax plus], (T21) vitamin C, (T22) [*T. harzianum* + vitamin C], (T23) *T. harzianum*, (T24) Rizolex-T 50WP and (T25) untreated control

Table 3 SDS-PAGE electrophoretic-gram data analysis for effect of *Trichoderma harzianum*, some salts and antioxidants on protein pattern of faba bean plants against root rot infection

Band No	RF	M. Wt	Bands intensity of different treatments									
			Vitamin E + vitamin C + enervit + selenium + vitamax plus		*Trichoderma harzianum* + vitamin E + vitamin C + enervit + selenium + vitamax plus		*T. harzianum* (10^8 spores/ml)		Rizolex-T (50WP) (1 gm/l)		Untreated control	
			1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	0.044	160	–	–	616	485	405	–	702	618	730	356
2	0.076	148	2274	2086	–	410	682	–	–	441	422	427
3	0.096	140	–	–	–	–	–	–	566	567	–	–
4	0.111	125	–	–	–	–	459	545	–	–	457	332
5	0.235	80	–	1941	–	–	632	480	790	894	927	420
6	0.332	70	–	–	430	569	–	–	–	–	–	–
7	0.345	60	2277	1771	–	–	–	–	–	–	–	–
8	0.411	52	–	–	–	–	–	–	–	–	–	–
9	0.482	43	1791	2340	1705	1335	1463	1361	1070	651	644	799
10	0.513	30	2004	1237	530	726	1664	1465	1361	1176	–	–
11	0.599	28	2192	2035	770	448	–	–	1101	1095	953	801
12	0.685	20	1894	2032	–	–	834	728	–	–	795	421
13	0.724	18	–	–	674	674	–	–	1067	851	805	479
14	0.876	15	2398	–	430	430	370	701	697	705	581	722
No. of bands			7	7	7	8	8	6	8	9	9	9
			8		8		8		9		9	
New proteins after 2nd irrigation			1		1		–		1		–	
Deleted proteins after 2nd irrigation			1		–		2		–		–	
More intensive proteins in 2nd irrigation than 1st one			2		2		3		1		2	
More intensive proteins in 1st irrigation than 2nd one			4		3		3		5		6	
Proteins with same intensity after both irrigations			–		2		–		2		1	

Band No	RF	M. Wt	Bands intensity of different treatments									
			Disodium phosphate		Calcium sulfate		Potassium metabisulfite		Sodium sulfate		Cysteine	
			1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	0.044	160	–	–	–	–	–	–	–	–	–	–
2	0.076	148	–	–	–	–	–	–	–	–	–	–
3	0.096	140	–	–	–	–	–	–	–	–	–	–

Table 3 (continued)

Band No	RF	M. Wt	Bands intensity of different treatments									
			Disodium phosphate		Calcium sulfate		Potassium metabisulfite		Sodium sulfate		Cysteine	
			1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
4	0.111	125	–	–	–	–	–	–	–	–	–	–
5	0.235	80	671	–	–	1120	716	1179	798	1018	597	955
6	0.332	70	–	–	–	–	–	–	–	–	–	744
7	0.345	60	1144	–	–	1242	–	–	–	1055	–	–
8	0.411	52	1085	632	977	1659	–	–	1372	1260	868	1423
9	0.482	43	–	1641	1871	3163	1359	1755	–	1842	–	–
10	0.513	30	2431	1485	1480	1780	2344	2797	2034	2200	1607	1879
11	0.599	28	1720	1326	1123	2595	1632	2473	1466	1460	1084	1805
12	0.685	20	1540	–	–	2091	1479	1976	1784	942	827	1159
13	0.724	18	2081	–	–	–	–	1564	1075	1322	1527	1899
14	0.876	15	–	1509	2307	3379	2203	2664	–	969	–	–
No. of bands			7	5	5	8	6	7	6	9	6	7
			9		8		7		9		7	
New proteins after 2nd irrigation			2		3		1		3		1	
Deleted proteins after 2nd irrigation			4		–		–		–		–	
More intensive proteins in 2nd irrigation than 1st one			–		5		6		3		6	
More intensive proteins in 1st irrigation than 2nd one			3		–		–		2		–	
Proteins with same intensity after both irrigations			–		–		–		1		–	

Band No	RF	M. Wt	Bands intensity of different treatments									
			Potash alum		Cysteine + potash alum		Cysteine + *Trichoderma harzianum*		Potash alum + *T. harzianum*		Cysteine + potash alum + *T. harzianum*	
			1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	0.044	160	–	–	–	–	–	–	–	–	–	–
2	0.076	148	–	–	–	–	–	–	–	–	1601	1754
3	0.096	140	–	–	–	–	–	–	–	–	–	–
4	0.111	125	1895	1594	–	–	2056	1646	1756	1639	–	–
5	0.235	80	–	–	1449	3574	–	–	–	–	1159	1524
6	0.332	70	–	–	–	2585	–	–	–	–	–	–
7	0.345	60	–	–	1623	–	–	–	–	–	–	–
8	0.411	52	–	–	1954	3737	–	–	–	–	1212	2524
9	0.482	43	1837	1629	3334	3446	2130	2143	2370	1761	–	–

Table 3 (continued)

Band No	RF	M. Wt	Bands intensity of different treatments									
			Potash alum		Cysteine + potash alum		Cysteine + *Trichoderma harzianum*		Potash alum + *T. harzianum*		Cysteine + potash alum + *T. harzianum*	
			1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
10	0.513	30	–	–	2229	3916	–	–	–	–	–	–
11	0.599	28	–	–	2232	3114	4053	3644	3063	2162	1826	2393
12	0.685	20	2638	1926	–	–	1801	2519	1891	2102	2153	2251
13	0.724	18	1836	1451	2968	3254	1361	1759	2161	1752	1727	2743
14	0.876	15	1705	1556	–	2392	–	–	–	–	1290	2681
No. of bands			5	5	7	8	5	5	5	5	7	7
			5		9		5		5		7	
New proteins after 2nd irrigation			–		2		–		–		–	
Deleted proteins after 2nd irrigation			–		1		–		–		–	
More intensive proteins in 2nd irrigation than 1st one			–		6		2		1		7	
More intensive proteins in 1st irrigation than 2nd one			5		–		2		4		–	
Proteins with same intensity after both irrigations			–		–		1		–		–	

Band No	RF	M. Wt	Bands intensity of different treatments									
			Enervit		Selenium		Vitamin E		Vitamin C		Vitamax plus	
			1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	0.044	160	–	–	–	–	–	–	494	235	–	–
2	0.076	148	2360	2223	2490	1965	1346	2659	–	–	1984	2527
3	0.096	140	–	–	–	–	–	–	–	486	–	–
4	0.111	125	–	–	–	–	–	–	–	–	–	–
5	0.235	80	–	1779	1215	1787	–	1369	–	350	–	–
6	0.332	70	–	–	–	–	–	–	723	594	–	–
7	0.345	60	2397	2129	2250	2182	1505	2383	–	–	985	2242
8	0.411	52	–	–	–	–	–	–	–	–	–	–
9	0.482	43	–	–	–	–	2542	2092	1715	2217	2634	3103
10	0.513	30	2924	2922	1947	3628	1591	1785	765	2217	1199	2136
11	0.599	28	–	–	1967	2382	1692	2601	763	1374	2038	2355
12	0.685	20	2466	2205	1623	2320	1499	1966	–	–	2205	2595
13	0.724	18	2563	2285	–	–	–	–	846	598	–	–
14	0.876	15	2209	1998	2003	2311	1305	2755	704	633	2082	2755

Table 3 (continued)

Band No	RF	M. Wt	Bands intensity of different treatments									
			Enervit		Selenium		Vitamin E		Vitamin C		Vitamax plus	
			1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
No. of bands			6	7	7	7	7	8	7	9	7	7
			7		7		8		9		7	
New proteins after 2nd irrigation			1		–		1		2		–	
Deleted proteins after 2nd irrigation			1		–		–		–		–	
More intensive proteins in 2nd irrigation than 1st one				–	5		6		3		7	
More intensive proteins in 1st irrigation than 2nd one			5		2		1		4		–	
Proteins with same intensity after both irrigations			1		–		–		–		–	
Band No	**RF**	**M. Wt**	**Bands intensity of different treatments**									
			Trichoderma harzianum **+ enervit**		***T. harzianum*** **+ selenium**		***T. harzianum*** **+ vitamin E**		***T. harzianum*** **+ vitamin C**		***T. harzianum*** **+ vitamax plus**	
			1st	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**
1	0.044	160	–	–	–	–	–	–	621	565	376	502
2	0.076	148	592	766	517	859	–	688	–	–	284	–
3	0.096	140	–	611	–	–	–	–	730	582	–	–
4	0.111	125	–	–	–	494	581	587	–	–	–	–
5	0.235	80	–	771	–	658	–	541	498	444	–	–
6	0.332	70	–	–	–	–	–	–	1210	854	263	373
7	0.345	60	–	1390	954	777	537	912	–	–	–	–
8	0.411	52	505	–	–	–	–	–	–	–	–	–
9	0.482	43	410	729	–	–	–	–	2289	2022	1087	1211
10	0.513	30	448	2114	2670	1888	1988	2409	2289	941	708	499
11	0.599	28	503	1781	1012	1594	1077	2566	1606	878	462	465
12	0.685	20	819	1538	1021	1326	1295	1773	–	–	–	–
13	0.724	18	750	736	1070	975	1040	1186	562	1222	819	826
14	0.876	15	479	1486	1626	1241	1672	1425	1292	1294	345	410

Table 3 (continued)

Band No	RF	M. Wt	Bands intensity of different treatments									
			Trichoderma harzianum + enervit		*T. harzianum* + selenium		*T. harzianum* + vitamin E		*T. harzianum* + vitamin C		*T. harzianum* + vitamax plus	
			1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
No. of bands			8	10	7	9	7	9	9	9	8	7
			11		9		9		9		8	
New proteins after 2nd irrigation			3		2		2		–		–	
Deleted proteins after 2nd irrigation			1		–		–		–		1	
More intensive proteins in 2nd irrigation than 1st one			7		4		6		1		4	
More intensive proteins in 1st irrigation than 2nd one			–		3		–		7		1	
Proteins with same intensity after both irrigations			1		–		1		1		2	

In the present study, applications of individual cysteine or combined with potash alum or *T. harzianum* as seed dressing, followed by two supported irrigations revealed a reduction in root rot disease incidence. In this regard, the in vitro effects of cysteine on fungal growth have been reported to inhibit spore germination of some fungal weed pathogens as *Alternaria* species that may be helpful in prohibiting fungal diseases caused by other fungal pathogens (Daigle and Cotty 1991). The foliar spray of bio-stimulator, *T. harzianum* with the chemicals; cysteine, calcium sulphate, disodium phosphate, potash alum, potassium metabisulfite and sodium sulfate greatly reduced the incidence and severity of faba bean rust disease (El-Mougy and Abdel-Kader 2018). Application of potassium sorbate, sodium benzoate, sodium metabisulfite, ammonium carbonate, ammonium bicarbonate and potassium benzoate alone or combined together efficiently alleviated the root rot in kiwifruit (Turkkan 2015). The effectiveness of antioxidants utilized in present study could be attributed to their chemical constants of vitamins used. It was reported that antioxidants are vital to raise host plant resistance, trigger innate immunity in plants and promote plant defence methods against the pathogens (Madukwe et al. 2013).

In this study, the resulted data showed that, the incidence of faba bean root rot at pre- and post-emergence growth phases was remarkably reduced under the seed dressing effect of some inorganic salts and antioxidants application individually or in combination with each other or with the bio-stimulator *T. harzianum,* followed by two successive irrigations with the same treatments. The application of these inducers exceeded the utilized fungicide, Rhizolex-T for the same aim. The 1st irrigation with enervit after seed dressing induced the highest protective effect against faba bean root rot pathogens invasion (5.0% incidence) during the pre-emergence stage. The 2nd irrigation with all treatments at post-emergence stage completely suppressed 100% of root rot incidence except vitamax plus (incidence of 91.8%).

Plant responds to pathogens infection via stimulating the expression of several proteins and enzymes. Proteins have important functions for plant defense responses, since structural proteins strengthen and repair plant cell walls or modify the properties of the extracellular matrix. Other proteins exhibit antimicrobial activities that catalyze the synthesis of various antimicrobial molecules (Souza et al. 2017). The antioxidant enzymes of plant (superoxide dismutase, CAT, POD, glutathione peroxidase and ascorbate peroxidase) work as part of the plant defense system and form a complex set of mechanisms to alleviate, neutralize and scavenge the reactive oxygen species (ROS). Identifying antioxidant enzymes activities is essential to understand the defensive mechanism systems of plant, as they play crucial roles in signal transduction and scavenging ROS in different compartments of the cell and in response to various infections (Nasr-Eldin et al. 2019). Also, plant CHIAs are part of pathogenesis-related proteins that are strongly expressed under infection stress for playing critical roles against fungal pathogens (Vaghela et al. 2022).

In the present study, all treatments after both irrigations induced the synthesis of different protein contents in faba bean plants than the healthy untreated control. All treatments after 2nd irrigation time induced higher protein content than 1st one, except three treatments [*T. harzianum* + vitamin E + vitamin C + enervit + selenium + vitamax plus], [*T. harzianum* + vitamax plus] and cysteine. Catalase, peroxidase and chitinase activities were increased by some treated faba bean plants after both irrigations. Some treatments induced higher CAT, POD and CHIA specific activities after the 2nd irrigation than the 1st one, while others induced lower specific activities. Disodium phosphate induced the highest CAT specific activity after both irrigations. [*T. harzianum* + vitamax plus] induced the highest POD specific activity after 1st irrigation time, while vitamin E induced the highest POD specific activity after 2nd one. Also, disodium phosphate induced the highest CHIA specific activity after 1st application, while [*T. harzianum* + vitamin E + vitamin C + enervit + selenium + vitamax plus] induced the highest CHIA specific activity 2nd one. Obtained data analysis of SDS-PAGE protein patterns of different treated faba bean plants with various inducers showed that different molecular weight proteins ranged from 160 to 15 kDa were expressed on treating with various treatments. The application with 10 inducers (calcium sulfate, potassium metabisulfite, sodium sulfate, cysteine, enervit, vitamin E, [*T. harzianum* + selenium], [*T. harzianum* + vitamin E], [*T. harzianum* + vitamin E + vitamin C + enervit + selenium + vitamax plus] and vitamin C) enhanced the formation of new protein bands after 2nd irrigation time that were not formed after the 1st one. The two treatments [*T. harzianum* + vitamax plus] and *T. harzianum* formed new protein bands after 1st irrigation time that were decomposed after the 2nd one. There are other 4 treatments; disodium phosphate, [cysteine + potash alum], [vitamin E + vitamin C + enervit + selenium + vitamax plus] and [*T. harzianum* + enervit] cause both effects but at different molecular weights. Applications with calcium sulfate, potassium metabisulfite, cysteine, [cysteine + potash alum], [cysteine + potash alum + *T. harzianum*], vitamin E, vitamax plus and [*T. harzianum* + enervit] increased the intensity of the proteins with the same molecular weight after 2nd irrigation time rather than the 1st one.

Conclusions

It was concluded that, the, 2nd irrigation time increased the efficiency of the treatment with the used inducer and became preferable in resistance and the formation of new resistance proteins (new protein genetic markers) against the root rot pathogens. Therefore, it is recommended to use these treatments with repeated applications more than once during the faba bean growing season to raise the plant's ability to resist disease and protect it until the end of the cultivating season. Thus, the future use of such treatments on a commercial scale for controlling root rot disease is promising, since the treatments used in this research gave good results compared to the recommended traditional fungicide. So, they could be considered good and environmentally safe alternatives for sustainable resistance against root system diseases and getting rid of the negative effects of fungicides.

Abbreviations

CAT	Catalase enzyme
POD	Peroxidase enzyme
CHIA	Chitinase enzyme
SDS-PAGE	Sodium dodecyl-sulfate polyacrylamide gel electrophoresis

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Author contributions

All authors contributed to the experimental design, hands on work, discussions, analyzed data, wrote the manuscript, reviewed the manuscript and approved the final manuscript.

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Availability of data and materials

All created and/or analyzed data during the present study are attainable in the manuscript, and the corresponding author has no interception to the availability of data and materials.

Declarations

Ethics approval and consent to participate

The study was conducted on natural occurrence of root rot disease and usage of beneficial fungi and certain plant resistance inducers that are available in the environment and the ethical approval is not demanded.

Consent for publication

Not Applicable.

Competing interests

All authors declare that they have no conflict of interest.

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