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Amelioration of the Structural and Biochemical Features of Kidney Bean against Root Rot and Rust Diseases

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ABSTRACT

The influential fabaceous crop; *Phaseolus vulgaris* L. is subjected to destructive infection by root rot and rust diseases, causing serious damage to productivity. The possible role of gibberellic acid (GA_3), paclobutrazol (PBZ), and abscisic acid (ABA) in improving the structural and biochemical resistance against such kidney bean diseases were studied. Pathogenicity test indicated that all tested root rot pathogenic fungi caused symptoms of damping-off. *Pythium ultimum* was the most aggressive in pre-emergence damping-off while *Fusarium oxysporum* gave the highest values of post-emergence damping-off and seedlings mortality. PBZ and ABA showed fungicidal activity against mycelia growth of root rot fungi. The high concentration of PBZ (150 ppm) was the most effective. PBZ and ABA significantly reduced the root rot and rust diseases, but GA_3 induced both diseases. Botanically, GA_3 (150 ppm) maximized plant height, and (PBZ 100 ppm) maximized branches number. Biochemically, both PBZ and ABA increased chlorophyll content, carotenoids, total phenolics and total soluble carbohydrates except the high level of ABA (15 ppm), which gave opposite results. On the other hand, GA_3 decreased chlorophyll content, phenols and total soluble carbohydrates. With regard to enzymes activity GA_3, PBZ and ABA induced the activity of peroxidase enzyme while reduced polyphenol oxidase activity. Anatomically, infected roots characterized by complete distraction changes in the epidermis and separation of some cortex tissue area. On the other side, all tested treatments decreased the harmful effects of pathogen fungi on root structure. In this respect, PBZ gave the highly effect. PBZ and ABA increased the thickness of leaflet and mesophyll tissue. So also, observed increase on stem diameter, cortex thickness, number of cortex cell layers and phloem fibers thickness. The application of PBZ at 100 ppm maximized the number of plant pods. GA_3 at 100 ppm gave the highest 100-seed weight. Therefore, treatments with either PBZ (100 ppm) or ABA (10 ppm) is recommended for alleviation the harmful effects of root rot and rust diseases in kidney bean.

Keywords: Structural resistance, biochemical resistance, beans, growth regulators, anatomy

INTRODUCTION

Kidney bean (*Phaseolus vulgaris* L.) is the most important economic and vital fabaceous crops in Egypt. It is providing high-quality protein for human consumption and has beneficial effects on soil fertility (Anonymous, 2005). Also, it is considered one of the most important vegetable crops that occupies a great figure in export (Mahmoud *et al.,* 2013).

Damping-off, root rot and wilt diseases are the most damaging soil and seed-borne diseases attack kidney bean, which affect germination, plant growth and yield. These diseases caused by several complex fungi such as *Rhizoctonia solani*, *Fusarium solani*, *F. oxysporum* and *Pythium* spp. (El-Shami,2008, Abd-El-Khair *et al.,* 2011 and Mahmoud *et al.,* 2013). *Rhizoctonia* root rot caused by *Thantephorus cucumeris* (*Rhizoctonia solani*) is common throughout the world under soil temperature of 18 ºC and normal soil moisture, causing severe disease. The symptoms infection is reddish-brown, elongate, cankerous lesions present on lower hypocotyl and root. Brown sclerotia develop on cankers. The young plants diseased show red coloration to the pith and may die. *Fusarium* spp, cause *Fusarium* root rot and spread by drainage or irrigation water (Hagedorn and Inglis, 1986). They also stated that, *Pythium ultimum* and some nematodes enhance disease severity of *Fusarium* root rot. Disease symptoms are small, elongate, and tan-red lesions in the upper taproot and lower hypocotyl, diseased plants are stunted and finally died. Pythium root rot caused by several species of *Pythium*, the most common is very likely *P. ultimum* occurs worldwide. The disease symptoms are elongate water-soaked areas on root and hypocotyls. The water-soaked areas become slightly sunken, tannish-brown lesions which coalesce giving the entire root system and lower stem collapsed, shrunken, tan-brown appearance because of the soft rot.

Bean rust disease occurs worldwide, leading to losses range of 13-100% when plants infected during the pre-flowering and flowering stages. *Uromyces appendiculatus* (*Uromyces phaseoli*) causes bean rust disease, which affects leaves and sometimes stems, petioles and pods. The symptoms of rust appear on the under surface of leaves as tiny, white, raised spots, the spots gradually enlarge and form reddish-brown pustules, which eventually erupt to release rusty masses of spores (Ryerson *et al.,* 1993).

Activation of plant resistance or induced disease resistance dependent on the host plants physical or chemical barriers, which activated by biotic and abiotic agents (Walters *et al.,* 2007). Physical (structural) resistance includes the thickness of cell wall and epidermis in different plant organs, which act as a mechanical barrier against invasion of the pathogen. In addition, structural polysaccharides of plant cell wall *i.e.* cellulose, hemicellulose and pectin, which play an important role in plant defense. Moreover, biochemical resistance (chemical barriers) includes phenols and activation of many enzymes such as peroxidase and polyphenol oxidase (Abd-El-Khair *et al.,* 2011).

Several reports indicated the efficiency of growth regulators in reducing fungal disease infection in many plants. In this respect, gibberellic acid (growth promoter) on peanut root rot (Metwally *et al.,* 2006), paclobutrazol (growth retardants) on peanut root rot (Fouda and Abdalla, 2000) and abscisic acid (growth inhibitors) on bean rust (Ryerson *et al.,* 1993).

The purpose of this study was to improve the structural and biochemical resistance of *Phaseolus vulgaris* against root rot and rust diseases using some types of growth regulators (GA3, PBZ and ABA).

MATERIALS AND METHODS

Kidney bean seeds and chemicals

Bean seeds (cv. Giza 3) were obtained from Vegetable Crops Research Department, Agric., Res., Centre, Giza, Egypt. While, Gibberellic acid 92% (GA3;

Berelex taplets, Imperical Industries Plc, plant protection division “ICI, England) was used as a growth promoter. More, Paclobutrazol (PBZ; 2Rs -3RS-1-(4- chlorophenyl)-4,4- dimethyl -2- (1,2,4-triazal -1-yl)- pentan -3-ol) was used as a growth retardant. In addition to, Abscisic acid (ABA; 1- deoxy -1,4- trans-diol, α- ionylidene ethanol) used as a growth inhibitor, as well as Topsin M 70% WP as a fungicide of root rot and plantvax 20% EC as a fungicide of rust disease were obtained from Sigma Company.

Root rot pathogens

Naturally infected bean plants, showing damping off and root rot symptoms collected from different fields in Dakhlia Governorate, Egypt. The infected roots washed thoroughly with tap water, cut into small pieces and surface-disinfected for two min. with 2% sodium hypochlorite, then re-washed in sterilized distilled water and dried with sterilized filter papers. Samples placed onto PDA medium in petri dishes supplemented with streptomycin-sulphate (100 mg ml^{-1}) and incubated at 25 ± 2°C for five days. The growing fungi were isolated and purified using the hyphal-tip and single spore techniques.

The selected fungi identified based on their cultural, morphological properties, taxonomic criteria and microscopic characteristics (Ellis 1976, Booth 1977, Sneh *et al,* 1992 and Sime *et al.,* 2002).

Pathogenicity test

Pathogenicity tests of isolated and identified fungi (*F. oxysporum, F. salami, R. solani* and *P. ultimum*) on bean plants were determined under the greenhouse. An artificial inoculum of each pathogenic fungus was prepared by growing it on sorghum – sand - water (2-1-2 v/v) medium. The contents of media were mixed, bottled, autoclaved and inoculated using agar discs obtained from the periphery of 5 day-old colony of the isolated fungi. Inoculated media incubated at 25 ± 2°C for 15 days. The inoculum of each pathogen mixed thoroughly with the autoclaved clay soil at the rate of 2% w/w. The infested soil put in sterilized pots (25 cm diameter). The infested pots irrigated and kept for 7 days before sowing. Three replicates used in each treatment beside check treatment (uninfected soil). Healthy bean seeds were surface sterilized and sown at the rate of 10 seeds pot^{-1}. At 15 and 30 days after sowing, seedlings rated for pre- and post-emergence damping-off, respectively.

Greenhouse experiment

Effect of growth regulators on soil-borne fungi

A mixture pathogenic fungi composed of an equal amount of each fungal inoculum was mixed with soil surface in plastic bags at the rate of 2% (w/w) under the greenhouse. Before this, an antagonism tests were carried out to insure that there is not any antagonism among tested pathogenic fungi. Infested bags irrigated and kept for 7 days before sowing. Bean seeds were soaked for 2 hr in three tested growth regulators; GA_3 at 50, 100 and 150 ppm, PBZ at 50,100 and 150 ppm and ABA at 5, 10, 15 ppm and sown in infested soil at the rate of 6 seeds/bag. The fungicide Topsin M 70% WP used as seed coating at the rate of 3 g kg^{-1} seeds. Percentage of pre-and post-emergence damping-off were recorded at 15 and 30 days after sowing, respectively. The percentage of root rot and wilt diseases were assessed at 60 days from sowing.

Effect of tested growth regulators on fungal growth

The tested growth regulators added individually to conical flasks containing sterilized PDA medium before solidification to obtain 50,100 and 150 ppm for GA_3 and PBZ as well as 5, 10 and 15 ppm for ABA and 3 g L^{-1} for the fungicide Topsin M 70% WP. Ten ml of the supplemented media of each treatment poured into petri dishes (9 cm diameter). The plates inoculated with mycelia discs (5 mm) at the center and incubated at 25 °C. Three replicates used for each treatment. In addition, three replicates were prepared to serve as control for each fungus. Fungal growth measured when the full growth of tested fungi observed under any treatment used and the average growth diameter was calculated.

Field experiment

A field experiment carried out at Tag El-Ezz Agricultural Research Station Farm, Dakahlia Governorate, Egypt during 2017 growing season under natural infection. Bean seeds were soaked individually in each concentration of the tested growth regulators before sowing and sprayed after 30 and 45 days during vegetative growth with the same treatments. Topsin M 70% WP applied only as seed coating at the recommended rate (3g kg-1 seed) for controlling damping-off and root rot diseases (as check treatment). While plantvax 20% EC used as a foliar spraying at 30 and 45 days after sowing for controlling rust disease (as check treatment). The experiment consisted of plots (3 X 3.5 m) contains 6 rows and 15 holes row-1. Complete randomized block design with three replicates was used for each treatment and untreated check treatment. Bean seeds (treated and untreated) sown in March 13th at the rate of 2 seeds hole-1. The average percent of damping-off, as well as dead plants, had recorded after 30 and 60 days from sowing, respectively.

Rust disease

Disease severity and disease incidence (infected %) of bean rust were recorded at 60^{th} day from sowing according to the standard scale (1-9) suggested by Bernier *et al.,* (1993). Next, rust disease severity % was calculated using the following formula:

$$\text{Disease severity \%} = \frac{\Sigma(NPC \times CR)}{(NIP \times MSC)} \times 100$$

Where NPC = No. of plants in each class rate

CR = class rate

NIP = No. of infected plants.

MSC = Maximum severity class rate.

While disease incidence (DI %) was calculated according to the following formula:

$$\text{Disease incidance} = \frac{\text{Number of infected leaflets}}{\text{Total number of tested leaflets}} \times 100$$

Growth and physiological parameters and yield

Samples of bean were taken at the 60^{th} day of the cultivation to determine growth parameters (plant height and branches $no.plant^{-1}$), as well as, physiological activities, including; (1) photosynthetic pigments (chlorophyll a, b and carotenoids), which were extracted from leaflet blade by methanol 90% and determined spectrophotometrically at wave lengths of 452.5, 650 and 665 according to Mackinney (1941**),** (2) Total phenolics that were determined in fresh shoot with the Folin-Ciocalteau reagent according to Malik and Singh (1980) and (3) total soluble carbohydrates were extracted by ethanol 70% in dry shoot (Kayani *et al.,* 1990) and determined by Anthron method according to Sadawivam and Manikam (1996). Samples were taken at harvesting to estimate the number of pods plant^{-1} and weight of 100 seeds.

Enzymatic assay

Peroxidase and polyphenoloxidase assay: The leaflet enzymes extract was prepared by ground the leaflet tissues with 0.1 M sodium phosphate buffer at pH 7 as a mortar. Through four layers of cheesecloth; the ground tissues were filtered and the filtrates were centrifuged at 3000 rpm for 20 min at 6^{o}c. Then peroxidase and polyphenol oxidase activity were determined according to Kato and Shimizu, (1987) and Esterbaner *et al.,* (1977), respectively

Anatomical studies

The specimens of bean roots were taken from the primary root (5-7 cm under soil surface) at 30 days after sowing in infested soil under greenhouse to determine the anatomical changes in kidney bean root due to the complex soil-borne fungi and growth regulators. In addition to, specimens from stem (2nd internode below the shoot tip) and in the midrib of the terminal leaflet were taken after 60 days from sowing under field condition. The specimens were fixed in FAA solution, dehydrated in alcohol series followed by xylene for cleaning and embedded in paraffin wax. Cross sections at 12-14Mm thick were prepared using rotary microtome, stained with crystal violet and erythrosine, cleared in clove oil and mounted in Canada balsam (Gerlach, 1977). The cross sections were examined microscopically.

Statistical analysis

The experiments were arranged in one-way randomized blocks, obtained data were analyzed using CoStat software (version 6.4). The analysis of variance was applied and comparison among different means was carried out according to Duncan multiple range test, at probability level ≤ 0.05.

RESULTS

Root rots fungal isolation and their pathogenicity test

Three fungal genera include four species (*F. oxysporum, F. solani, R. solani and P. ultimum*) were isolated from naturally infected bean plants showing root rot symptoms. The isolated fungi identified and tested for their pathogenic capability on cv. Giza3 under greenhouse conditions. Data in Table 1 show that, the four tested fungi were pathogenic and caused pre-and post-emergence damping-off on bean seedlings. *P. ultimum* followed by *R. solani* were the most aggressive pre-emergence damping-off causatives. While, *F. oxysporum* give the highest percentage of post-emergence damping-off followed by *F. solani* and *R. solani*. Moreover, *F. oxysporum* showed to be the most causative fungus in seedlings mortality followed by *F. solani* then *P. ultimum*.

Growth regulators against fungal growth

The activities of growth regulators against the mycelial growth of the tested root rots fungi were evaluated. As could be observed in Table 3, Topsin M 70% WP and high concentrate of PBZ (150 ppm) prevented the growth of all tested fungi. Meanwhile, GA_3 led to a significant increase in fungal growth. *P. ultimum* was the most sensitive for growth regulators followed by *F. solani* while *R. solani* was the least affected.

Table 1. Pathogenicity test of root rot fungi on kidney bean plants

Fungus	Pre-emergence	Post-emergence	Survival
F. oxysporum	25.67 c	41.67 a	32.67 c
F. solani	23.67 c	39.67 a	36.67 c
R. solani	30.33 b	20.67 b	49.00 b
P. ultimum	35.33 a	16.67 c	48.00 b
Check	1.00 d	0.00 d	99.00 a

Means within each column followed by different letter significantly differ.

Effect of growth regulators on tested soil-borne fungi

The efficacy of growth regulators and Topsin M 70% WP for controlling damping-off had evaluated in infested soil with a mixture of pathogenic fungi under greenhouse condition. Table 2 show that, Topsin M 70% WP presented the highest reduction in pre-and post-emergence damping-off as well as the % of root rot and wilt diseases. While GA_3 gave an opposite effect and increased with increasing the concentration of GA_3. On the other hand, both growth retardant (PBZ) and growth inhibitor (ABA) gave a significantly reducing of bean damping-off, as well as dead plants (resulted from root rot and wilt). The maximum reduction recorded by ABA at 15 ppm followed by ABA at 10 ppm and PBZ at 150 ppm.

Table 2. Effect of growth regulators on damping-off, root rot and wilt on kidney bean under artificially infested soil

Treatment	Pre-emergence	Post-emergence	Root rot%	Wilt %
Mixture fungi	19.00 b	16.00 b	30.00 c	13.67 c
Topsin M	2.67 g	2.00 g	8.00 i	1.67 i
GA_3 50 ppm	20.67 a	17.00 a	32.00 b	16.00 a
GA3 100ppm	19.67 b	16.00 b	30.00 c	13.00 c
GA_3 150ppm	21.67 a	18.00 a	34.33 a	18.00 a
PBZ 50 ppm	12.00 c	10.67 c	22.00 d	8.00 d
PBZ 100 ppm	9.33 d	9.00 d	18.67 e	6.00 e
PBZ 150 ppm	8.00 e	6.67 e	16.00 f	5,00 f
ABA 5 ppm	10.67 cd	10.00 cd	19.00 e	7.33 e
ABA 10 ppm	7.67 e	6.00 e	14.00 g	4.00 g
ABA 15 ppm	5.00 f	5,00 f	11.00 h	3.00 h

Means within each column followed by different letter significantly differ.

Table 3. Effect of growth regulators on linear growth (cm) of the tested pathogenic fungi

Treatment	*F. oxysporum*	*F. solani*	*R. solani*	*P. ultimum*
Check	7.87 c	7.67 c	8.47 b	7.73 c
Topsin M	0.0 i	0.0 i	0.0 h	0.0 i
GA_3 50 ppm	8.6 b	8.27 b	9.0 a	8.2 b
GA_3 100 ppm	9.0 a	9.0 a	9.0 a	9.0 a
GA_3 150 ppm	9.0 a	9.0 a	9.0 a	9.0 a
PBZ 50 ppm	3.5 f	2.83 f	3.63 e	2.73 f
PBZ 100 ppm	1.47 h	1.23 h	1.77 g	1.07 h
PBZ 150 ppm	0.0 i	0.0 i	0.0 h	0.0 i
ABA 5 ppm	5.1 d	5.0 d	5.53 c	4.13 d
ABA 10 ppm	4.67 e	4.17 e	4.67 d	3.53 e
ABA 15 ppm	2.47 g	2.1 g	2.97 f	2.00 g

Means within each column followed by different letter significantly differ.

Effect of growth regulators under field conditions

On Root rots diseases

The effect of the three tested growth regulators, as well as Topsin M 70% WP on the percentage of damping-off and dead plants in field under natural infection, presented in Table 4. Data show that, the application of PBZ and ABA, as well as Topsin M 70% WP, significantly decreased emergence damping-off and dead plants. PBZ at 100 ppm was the most effective in reducing of damping-off and dead plants compared with other growth regulators. While, GA_3 significantly increased damping-off and dead plants.

Table 4. Effects of growth regulators on damping-off and dead plants (root rot and wilt) on kidney bean under field conditions.

Treatment	Damping-off	Dead plant
Control	18.00 c	24.67 b
Topsin M	1.67 h	3.67 e
GA3 50 ppm	17.33 c	24.33 b
GA3 100 ppm	22.33 b	30.00 a
GA3 150 ppm	28.00 a	32.33 a
PBZ 50 ppm	10.00 ef	13.67 c
PBZ 100 ppm	5.67 g	6.67 d
PBZ 150 ppm	8.33 fg	9.33 d
ABA 5 ppm	13.00 d	15.67 c
ABA 10 ppm	11.33 de	14.67 c
ABA 15 ppm	13.00 d	15.67 c

Means within each column followed by different letter significantly differ.

On Rust disease

Data in Table 5 show that, soaking bean seeds in combined with foliar spraying at 30 and 45 days with PBZ and ABA or foliar spraying with plantvax 20 % EC decreased significantly severity and incidence of rust disease. PBZ at 100 ppm give the highest effect in reducing of rust disease severity followed by ABA at 10 ppm compared with other growth regulators. Plantvax 20 % EC followed by ABA at 5 ppm and PBZ at 50 ppm give the greatest inhibition of disease incidence. On the other hand application of GA_3 significantly increased rust disease severity.

Morphological evaluation

Data concerning the average plant height and number of branches per plant in relation to the growth regulators presented in Table 6. It could be observing that, all levels of GA_3 significantly increased plant height while PBZ and ABA give reverse effects. These effects increased with the increment of the tested concentration. The highest values of branches number recorded with PBZ at 100 ppm while GA_3 at 150 ppm give the lowest values. While, the both of fungicides did not give any significantly impact on development parameters.

Physiological features

Photosynthetic pigments content

The three levels of GA_3 significantly decreased chl a & b (Table 7). On the other hand, PBZ and ABA significantly increased chlorophyll and carotenoids content except for the high level of ABA, which decreased them. While, there is any significant effect in the total chlorophyll in bean leaflets under both fungicides application. PBZ at 100 ppm gave the highest carotenoids content followed by ABA *at 5 ppm.*

Table 5. Effects of growth regulators on rust disease on kidney bean under natural field conditions.

Treatment	Disease severity	Disease incidence
Control	76.33 b	36.67 c
Plantvax	7.0 h	3.67 h
GA_3 50 ppm	86.67 a	41.30 b
GA_3 100 ppm	89.0 a	45.67 a
GA_3 150 ppm	74.67 b	35.00 c
PBZ 50 ppm	17.33 ef	7.33 fg
PBZ 100 ppm	9.33 gh	10.00 f
PBZ 150 ppm	29.33 d	18.33 d
ABA 5 ppm	20.67 e	6.67 g
ABA 10 ppm	13.33 fg	8.33 fg
ABA 15 ppm	37.33 c	14.33 e

Means within each column followed by different letter significantly differ.

Table 6. Effects of growth regulators on plant height and branches number of kidney bean plants.

Treatment	Plant height (cm)	Branches no. $plant^{-1}$
Control	47.00 d	2.67 cd
Topsin M	46.00 d	2.67 cd
Plantvax	48.33 d	3.0 c
GA_3 50 ppm	55.33 c	2.67 cd
GA_3 100 ppm	67.33 b	2.67 cd
GA_3 150 ppm	78.33 a	2.0 d
PBZ 50 ppm	41.00 e	4.0 b
PBZ 100 ppm	36.67 f	6.0 a
PBZ 150 ppm	29.33 g	2.67 cd
ABA 5 ppm	42.00 e	2.67 cd
ABA 10 ppm	38.33 ef	3.0 c
ABA 15 ppm	35.67 f	2.33 cd

Means within each column followed by different letter significantly differ.

Table 7. Effects of growth regulators on photosynthetic pigments (mg/g. F.W.) in kidney bean leaves.

Treatment	Chl. a	Chl. B	Total Chls	Carotenoids
Control	2.373 de	1.287 d	3.660 d	0.427 efg
Topsin M	2.383 de	1.323 cd	3.707 d	0.463 de
Plantvax	2.293 e	1.360 c	3.653 d	0.510 c
GA_3 50 ppm	1.947 f	0.973 f	2.920 e	0.477 cd
GA_3 100 ppm	1.750 g	0.870 g	2.620 f	0.410 fg
GA_3 150 ppm	0.930 h	0.657 h	1.587 g	0.250 h
PBZ 50 ppm	2.643 c	1.413 b	4.057 c	0.480 cd
PBZ 100 ppm	3.033 a	1.537 a	4.570 a	0.587 a
PBZ 150 ppm	2.433 d	1.330 c	3.763 d	0.443 def
ABA 5 ppm	2.840 b	1.540 a	4.380 b	0.550 b
ABA 10 ppm	2.400 de	1.320 cd	3.730 d	0.403 g
ABA 15 ppm	1.670 g	1.033 e	2.703 f	0.210 i

Means within each column followed by different letter significantly differ.

Phenol and carbohydrate content

PBZ and the both of fungicides as well as ABA, significantly increased phenol and carbohydrate content in bean shoot, except the high level of ABA at 15 ppm, which decreased these parameters (Table 8). Plantvax give the highest values of phenols followed by Topsin M 70% WP. Concerning the effects of growth regulators, the high phenols and total soluble carbohydrate recorded by PBZ at ppm. However, GA_3 at all concentrates decreased the aforementioned parameters.

Enzymatic activity

From Table 9 it is clear that, peroxidase activity increased greatly with application of fungicides, PBZ and ABA. PBZ was more effective followed the moderate levels of ABA. In contrast, GA_3 reduced the activity of peroxidase enzyme, especially at the higher concentration.

On the other side, polyphenol oxidase activity significantly increased under both of GA_3 and high level of ABA. While the other treatments decreased polyphenol oxidase activity as compared with control treatment.

Table 8. Effects of growth regulators on total phenol(mg/100 g. F.wt.) and total soluble carbohydrate(mg/g. dry wt.) in kidney bean shoots.

Treatment	Total phenol	Total soluble carbohydrate
Control	343.0 g	78.21 d
Topsin M	511.0 b	83.14 c
Plantvax	569.3 a	78.69 d
GA3 50 ppm	341.0 g	80.18 cd
GA3 100 ppm	323.0 h	72.02 e
GA3 150 ppm	309.7 h	70.04 e
PBZ 50 ppm	427.3 d	91.73 b
PBZ 100 ppm	490.0 c	105.85 a
PBZ 150 ppm	373.3 e	84.25 c
ABA 5 ppm	353.7 fg	89.76 b
ABA 10 ppm	368.3 ef	89.73 b
ABA 15 ppm	315.3 h	62.37 f

Means within each column followed by different letter significantly differ.

Table 9. Enzymatic activity (mg/g. F.wt.) of peroxidase and polyphenoloxidase in kidney bean as a response of growth regulators

Treatment	Peroxidase	Polyphenoloxidase
Control	0.332 gh	0.171 e
Topsin M	0.341 g	0.094 j
Plantvax	0.453 f	0.081 k
GA_3 50 ppm	0.325 gh	0.214 d
GA_3 100 ppm	0.314 h	0.228 c
GA_3 150 ppm	0.281 i	0.306 a
PBZ 50 ppm	0.551 c	0.112 i
PBZ 100 ppm	0.628 a	0.107 i
PBZ 150 ppm	0.523 d	0.121 h
ABA 5 ppm	0.528 d	0.149 f
ABA 10 ppm	0.586 b	0.136 g
ABA 15 ppm	0.488 e	0.284 b

Means within each column followed by different letter significantly differ.

Anatomical features

Root structure

Data in Table 10 show that, PBZ at all concentrates and ABA at 5 & 10 ppm as well as root rot fungi caused an increase in root diameter, cortex thickness and vascular cylinder diameter. PBZ at 150 ppm give the highest effect followed by PBZ at 100 ppm and ABA at 10 ppm. While, GA_3 at all concentrates give a reverse effect.

Table 10. Effect of fungal root rot and growth regulators on kidney bean root structure

Treatment	Root section		Cortex		Vascular cylinder	
	Diameter (µ)	%*	Thickness (µ)	%*	Diameter (µ)	%*
Check	1660	-	126	-	1270	-
Complex fungi	1970	18.67	168	33.33	1320	3.94
Topsin M	1520	-8.43	118	-6.35	1136	-10.55
GA_3 50 ppm	1420	-14.46	108	-14.29	1018	-19.84
GA_3 100 ppm	1310	-21.08	102	-19.05	1007	-20.71
GA_3 150 ppm	1290	-22.29	93	-26.19	983	-22.60
PBZ 50 ppm	2060	24.10	175	38.89	1410	11.02
PBZ 100 ppm	2340	40.96	196	55.56	1480	16.54
PBZ 150 ppm	2490	50.00	210	66.67	1510	18.90
ABA 5 ppm	1800	8.43	153	21.43	1295	1.97
ABA 10 ppm	2260	36.14	188	49.21	1450	14.17
ABA 15 ppm	1440	-13.25	114	-9.52	1052	-17.17

***Variation percent in relation to the control**

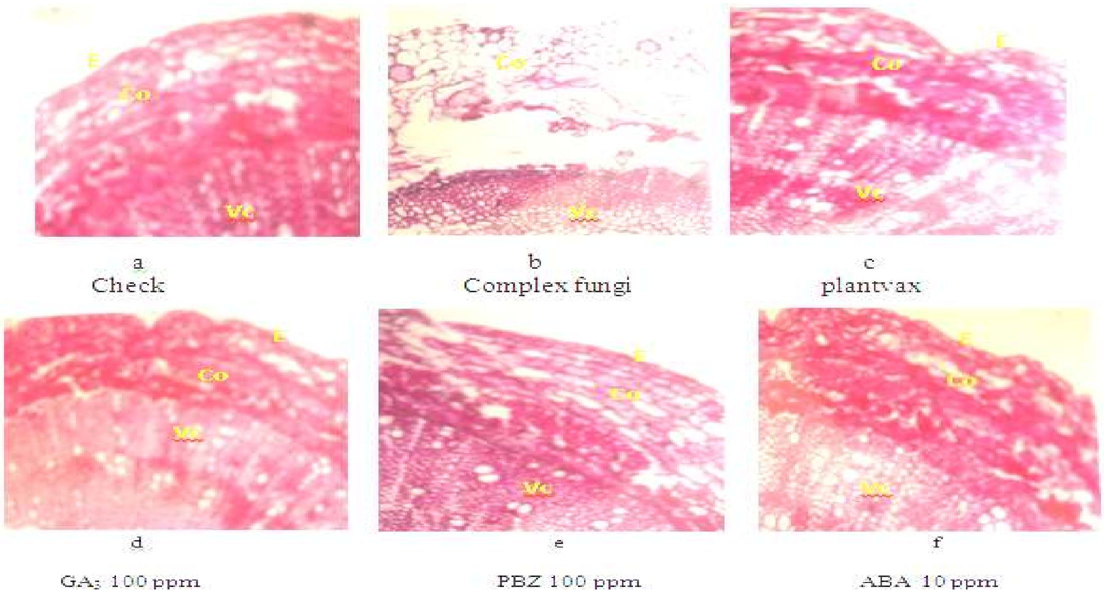


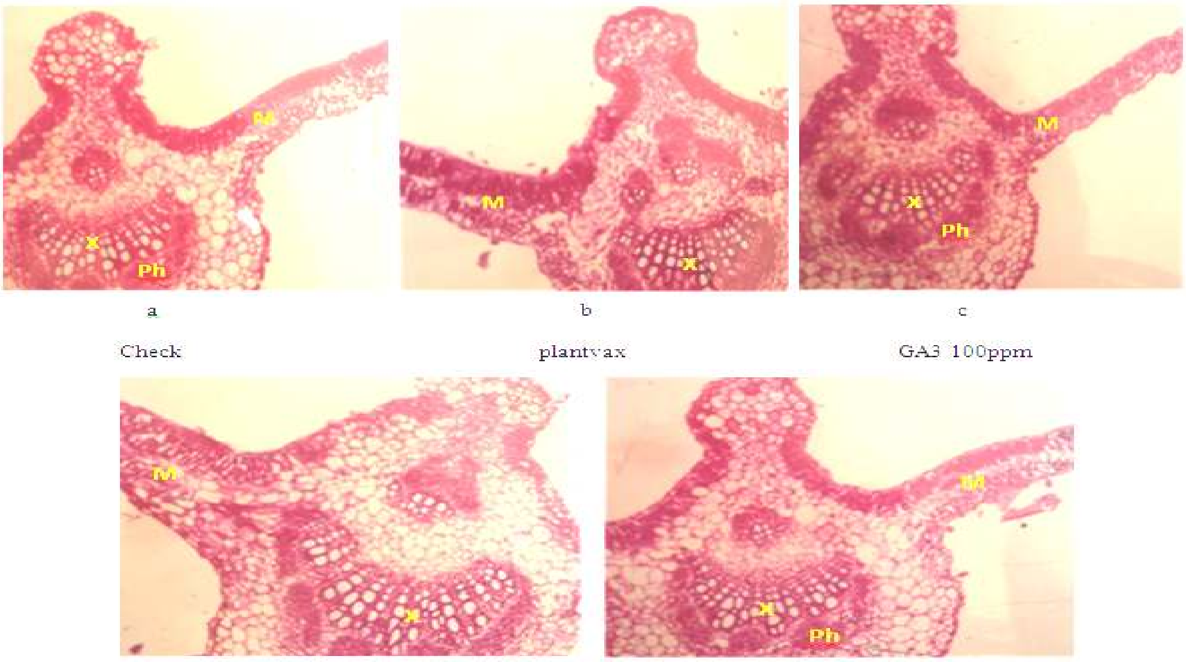
Fig 1. Effect of fungal root rot and growth regulators on kidney bean root structure (x 100)
E: Epidermis Co: Cortex Vc: Vascular cylinder

Data also show that, there are clear differences in root sections, which obtained from normal healthy plants (check), infected plants with complex root rots fungi, fungicidal treatment and growth regulators (Fig.1). The root cross-section of infected plants showed complete destruction of the epidermis, which exhibited brown epidermal surface. In addition, separation in some area of cortex tissue followed by degradation and dissolution of cell components were observed. On the other side, all of treatments led to decrease the harmful effects of root rot fungi on root structure.

Leaflet structure

Regarding to the anatomical structure of leaflet as influenced by various growth regulators (Fig 2 a, b, c, d, e), the normal bean leaflet structure shows a dorsiventral mesophyll having a uniseriate palisade parenchyma and few layers of loosely arranged chlorenchyma cells forming the spongy parenchyma. The vascular bundle of the midvein is an open collateral having a narrow zone of cambial initials (Fig 2 a).

Data presented in Table 11 show that, all concentrations of PBZ and ABA at 5 &10 ppm, as well as plantvax, increased the thickness of bean leaflet blade. These increases are due to a corresponding increase in the thickness of palisade and spongy tissues. In this respect, PBZ at 150 ppm gives the highest values followed by PBZ at 100 ppm and ABA at 10 ppm. On contrary, GA_3 at all concentrate and ABA at 15 ppm decreased these parameters. All tested treatments increased the midrib bundle dimensions as well as xylem and phloem tissue thickness except plantvax and ABA at 15 ppm.



a Check b plantvax c GA3 100ppm

d PBZ 100ppm e ABA 10ppm

Fig 2. Effect of growth regulators on kidney bean leaflet structure (x 100)

M: Mesophyll X: Xylem Ph: Phloem

Table 11. Effects of growth regulators on kidney bean leaflet structure.

Treatment	Leaflet		Palisade		Spongy		Diameter of the vascular bundle (µ)				Xylem tissue		Phloem tissue	
	Thickness (µ)	%*	Thickness (µ)	%*	Thickness (µ)	%*	Length	%*	Width	%*	Thickness (µ)	%*	Thickness (µ)	%*
Check	263	-	95.50	-	108.5	-	396	-	418	-	221	-	175	-
Plantvax	266	1.14	96.00	0.52	113.1	4.24	371	-6.31	420	0.48	213	-3.62	158	-9.7
GA_3 50 ppm	248	-5.70	90.00	-5.76	93.4	-13.91	430	8.59	627	50.00	246	11.31	184	5.14
GA_3 100 ppm	239	-9.13	82.70	-13.4	90.6	-16.5	442	11.17	720	72.24	260	17.65	182	4.00
GA_3 150 ppm	230	-12.55	80.50	-15.71	85.9	-20.83	418	5.56	622	48.80	233	5.43	185	5.71
PBZ 50 ppm	275	4.56	109.60	14.76	117.1	7.93	484	22.22	785	87.80	295	33.48	189	8.00
PBZ 100 ppm	286	8.75	117.20	22.72	125.3	15.48	520	31.31	810	93.78	327	47.96	193	10.28
PBZ 150 ppm	294	11.79	121.00	26.7	130.4	20.18	614	55.05	823	96.89	334	51.13	280	60.00
ABA 5 ppm	271	3.04	102.40	7.23	114	5.07	492	24.24	797	90.67	316	42.99	176	0.50
ABA 10 ppm	284	7.98	112.30	17.59	121.9	12.35	512	29.29	803	92.11	321	45.25	191	9.14
ABA 15 ppm	252	-1.18	92.10	-3.56	96.3	-11.24	384	-3.03	411	-1.67	218	-1.36	166	-5.14

***Variation percent in relation to the control**

Stem structure

Regarding to the anatomical structure of stem as influenced by various growth regulators (Fig 3 a, b, c, d, e), the structure of stem as shown from transverse sections of healthy bean plants consists of one layer of the epidermis, wide cortex tissue, phloem fiber groups and vascular collateral bundles arranged in complete cylinder. Two types of collateral bundles *i.e.* large and small bundles; large bundles are separated by few small bundles (Fig. 3 a).

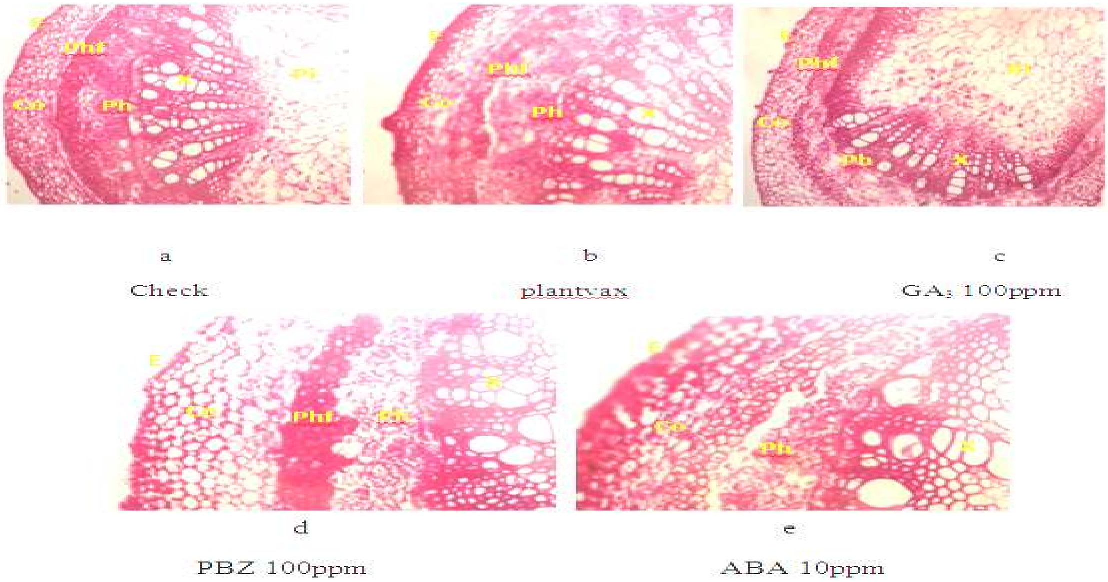


Fig 3. Effect of growth regulators on kidney bean stem structure (x100)
E: Epidermis Co: Cortex Phf: Phloem fiber Ph: Phloem X: Xylem Pi: Pith

All concentrate of PBZ, plantvax and ABA at 5&10 ppm led to increase of stem diameter, cortex thickness, number of cortex cell layers and phloem fibers thickness (Table 12). While, GA_3 at all concentrates and ABA at 15 ppm gives an opposite effect on these parameters except the effect of ABA at 15 ppm on phloem fibers thickness.

Table 12. Effects of growth regulators on kidney bean stem structure.

	Cross section		Cortex		Cortex cell layer		Phloem fibers	
Treatment	**Diameter (µ)**	**%***	**Thickness (µ)**	**%***	**Number**	**%***	**Thickness (µ)**	**%***
Check	5914	-	186	-	9	-	114	-
Plantvax	5919	0.08	193	3.76	10	11.11	118	3.51
GA3 50 ppm	5240	-11.4	180	-3.23	8	-11.11	112	-1.75
GA3 100 ppm	4890	-17.31	169	-9.14	7	-22.22	94	-17.54
GA3 150 ppm	4830	-18.33	158	-15.05	7	-22.22	90	-21.05
PBZ 50 ppm	6420	8.56	210	12.09	11	22.22	118	3.51
PBZ 100 ppm	6560	10.92	227	22.04	11	22.22	129	13.16
PBZ 150 ppm	7130	20.56	243	30.65	13	44.44	141	23.68
ABA 5 ppm	6075	2.72	198	6.45	10	11.11	119	4.39
ABA 10 ppm	6180	4.5	204	9.68	10	11.11	124	8.77
ABA 15 ppm	5430	-8.18	182	-2.15	8	-11.11	115	0.88

***Growth percent's more or less than the control**

Yield parameters

Data in Table 13 reveal that, growth regulators and fungicides give a significant increase in the number of plant pods except under ABA at 15 ppm. In this respect, PBZ at 100 ppm recorded the highest values followed by Plantvax, Topsin M and GA_3 at 100 ppm. Moreover, GA_3 at 100 ppm give the highest weight of 100-seed followed by PBZ at 100 ppm.

Table 13. Effects of growth regulators on number of pods/plant and weight of 100-seed on kidney bean plant.

Treatment	Pods no./plant	100-seed weight
Control	48.33 g	32.68 e
Topsin M	66.33 bc	32.76 e
Plantvax	70.33 b	32.94 e
GA3 50 ppm	60.00 de	36.28 b
GA3 1 00 ppm	66.00 c	38.80 a
GA3 150 ppm	57.00 ef	35.94 b
PBZ 50 ppm	63.00 cd	34.57 cd
PBZ 100 ppm	74.67 a	35.49 bc
PBZ 150 ppm	60.33 de	34.15 d
ABA 5 ppm	64.33 cd	35.49 bc
ABA 10 ppm	53.33 f	33.22 e
ABA 15 ppm	47.67 g	30.45 f

Means within each column followed by different letter significantly differ.

DISCUSSION

Kidney bean (*phaseolus vulgaris* L.) is one of the most important economic febaceous crops for local consumption and export in Egypt. The plant produces root nodules, which serve as replenishes of soil nitrogen, maintaining soil fertility and therefore reducing the need for artificial fertilizers. The production of sufficient crops is a major problem, which faces the developing countries all over the world. So, increasing the productivity considered as a national goal. Root rot and rust diseases are limiting factors of kidney bean planting, growth and productivity. Any factor causes increasing the structural and physiological resistance can protect the plants against pathogen infection. The present investigation concluded that all isolated soil-borne fungi were pathogenic and causative agents of bean damping off. The genus of *Fusarium* was the most virulent, causing seedlings mortality followed by *P. ultimum*. The harmful effects of the tested root rot fungi may be due to enzymes production, which causes rotten lesions on seed cotyledons, in turn; seed rot and plumule soft rot consequently pre -and post-emergence death (Mahmoud *et al.,* 2013). In this investigation, infested soil with the mixture of the pathogenic fungi causes injuries effects in root structure, which causes root rot and damping off.

In the present investigation, considerably that the concentrations of growth regulators were utilized at the minimum levels that expected to lie within the save level for human health. Both of growth retardant (PBZ) and of growth inhibitor (ABA), as well as fungicide (Topsin M), showed fungicidal activity against the mycelia growth of all tested fungi. Topsin M and PBZ at 150 ppm completely inhibited the growth of the four tested fungi. Fungal growth affected by PBZ due to inhibition of ergosterol biosynthesis that is essential for fungal membrane structure by adversely effects on the activity of membrane-bound enzymes (Mantecon, 2009). Treatment of bean seeds with Topsin M is requires in the germination stage to help and/or assure an adequate plant stand in the soil. Meanwhile, GA_3 as growth promoter stimulates mycelia growth of pathogenic fungi. This study showed clearly that both PBZ and ABA significantly reduced of bean damping off under the artificially infested soil with pathogenic fungi in the greenhouse and under natural infection in the field. The same effect had observed on rust disease severity and incidence under field condition. The beneficial effects of PBZ and ABA against root rot and rust diseases may be due to the inhibitory effects on fungal growth, increasing photosynthetic pigments, enhancing the level of plant phenols and carbohydrate content as well as increase the activity of peroxidase enzyme. Chlorophyll content is a good parameter reflecting the plant healthy. In addition, carotenoids considered had antioxidant effect, which protects chlorophyll from light oxidation and protects the plant from fungal stress. There is a positive relationship between photosynthetic pigments and carbohydrate content in plant organs. The carbohydrates comprise structurally polysaccharides in cell wall such as pectin, which acts as a barrier against pathogen invasion (Hamideh *et al.,* 2013).

It well known that, total phenolic compounds has highly antioxidants effect. However, total soluble carbohydrate is a good parameter reflecting positive health condition of the plant. Moreover, total phenols consider an antifungal compounds, which play an important role in plant protection against pathogens. On the other hand, peroxidase plays an important role in protect plants against pathogen infection. One of this role activities of plant resistance include oxidative cross-linking of pre-existing hydroxyproline-rich structural proteins in the cell wall, which making it more resistant to degradation by microbial enzymes, and it increases lignin production (Nawar and Kuti, 2003). Moreover, most of growth regulators led to increasing the total phenol, calcium content and catechol oxidase activity while reduced the activity of pectolytic enzymes (Chowdhury, 2003). In addition, Mauch-Mani and Mauch (2005) reported that, there is a positive correlation between ABA levels and disease resistance. Generally, our results proved that PBZ and ABA induced the structural resistance in root beans against root rot diseases in terms of increased root diameter and cortex thickness as well as stem diameter, cortex thickness, number of cortex cell and phloem fibres that act as a mechanical barrier against invasion of rust fungus. PBZ and ABA also induced the physiological resistance by activation of complex metabolic pathways. All of these process enhancing growth and productivity of kidney bean.

Therefore, it could be concluding that the application of PBZ (100 ppm) or ABA (10 ppm) consider a safe recommend for reducing root rot and rust diseases as well as improving the yield of bean plants compared to fungicides.

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تحسين الخصائص التركيبية والبيوكيميائية للفاصوليا ضد أمراض عفن الجذور والصدأ

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تتعرض الفاصوليا لإصابات خطيرة بأمراض عفن الجذور والصدأ والتي تسبب أضرارا عديدة للإنتاجية. تم دراسة الدور المحتمل للجبريللين والباكلوبترازول وحمض الأبسيسيك لتحسين المقاومة التركيبية والبيوكيميائية ضد أمراض الفاصوليا. أظهر اختبار القدرة المرضية أن كل فطريات عفن الجذور المختبرة سببت ظهور أعراض موت البادرات. وكان فطر *Pythium ultimum* الأكثر عدوانية في الإصابة بمرض موت البادرات قبل ظهورها فوق سطح التربة. بينما أعطى فطر *Fusarium oxysporum* أعلى قيم لموت البادرات بعد ظهورها فوق سطح التربة وكذلك البادرات الميتة. شوهد نشاط ابادى للباكلوبترازول وحمض الابسيسيك على النمو المسيليومى لفطريات عفن الجذور. وكان التركيز الأعلى للباكلوبترازول (١٥٠ جزء في المليون) هو الأكثر تأثيرا. أدت المعاملات بالباكلوبترازول وحمض الأبسيسيك الى نقص معنوى لأمراض عفن الجذور والصدأ في حين أن الجبريللين استحث الإصابة بكلا المرضين. نباتيا: أعطى الجبريللين (١٥٠ جزء في المليون) اعلى زيادة في طول النبات وأعطى الباكلوبترازول أعلى زيادة في عدد الأفرع. كيموحيويا: أدى كلا من الباكلوبترازول وحمض الابسيسيك الى زيادة محتوى الكلورفيل والكاروتنويدات والفينولات والكربوهيدرات الذائبة الكلية فيما عدا المستوى المرتفع لحمض الأبسيسيك (١٥ جزء في المليون) الذى أدى لنقص هذه الصفات. في المقابل أدى الجبريللين لنقص محتوى الكلورفيل والفينولات والكربوهيدرات الكلية الذائبة. وعلى عكس الجبريللين استحث الباكلوبترازول وحمض الأبسيسيك تنشيط انزيم البيروكسيديز وتقليل نشاط انزيم البولى فينول أوكسيديز. تشريحيا: شوهد تحطم كامل لنسيج البشرة مع حدوث انفصال لبعض المساحات بنسيج القشرة وذلك في القطاعات العرضية للجذور المصابة بفطريات عفن الجذور. وعلى الجانب الاخر أدت جميع المعاملات المستخدمة الى تقليل التأثيرات الضارة لفطريات عفن الجذور على التركيب التشريحى للجذر. وكان الباكلوبترازول هو الأكثر تأثيرا. وفى التركيب التشريحى للوريقات أدت معاملات الباكلوبترازول وحمض الأبسيسيك الى زيادة سمك الوريقة والنسيج المتوسط. وشوهد نفس التأثير على قطر الساق وسمك القشرة وعدد طبقات القشرة وسمك ألياف اللحاء في القطاعات العرضية للساق. محصوليا: أدى استخدام الباكلوبترازول ١٠٠ جزء في المليون لتعظيم عدد القرون في النبات و أعطى الجبريللين ١٠٠ جزء في المليون أعلى قيم لوزن ١٠٠ بذرة.ولهذا توصى الدراسة بالمعاملة بالباكلوبترازول بتركيز ١٠٠ جزء في المليون أو حمض الأبسيسيك بتركيز ١٠ جزء في المليون لتقليل التأثيرات الضارة لأمراض عفن الجذور والصدأ في الفاصوليا.