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Research Article
Improving the Performance, Productivity and Resistance of *Vigna radiate* to *Fusarium oxysporum* by Cytokinins and β-sitosterol

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Abstract

Background: *Fusarium oxysporum* causes vascular wilt diseases in a wide variety of economically important crops. Control of plant diseases still relies mainly on the use of synthetic fungicides, but environmental and health concerns and the development of fungicide-resistant pathogens have stimulated the search for alternative control strategies. **Materials and Methods:** *Fusarium oxysporum* isolated from the roots of diseased mung bean plans collected from Aga district, Dahahlia, Egypt (F_1) recorded the highest values of wilt disease incidence (50%) in the pathogenicity test. **Results:** Cytokinins at concentrations of 25, 50, 100 and 200 ppm and β-sitosterol at 10^{-1}, 10^{-3}, 10^{-5} and 10^{-7} M slightly reduced the linear growth of *F. oxysporum* on PDA solid medium. In the greenhouse experiment, the β-sitosterol at 10^{-5} M was the most effective treatment in reducing the incidence of wilt (11.38^{e}) in mung bean plant infected with *Fusarium oxysporum*. The growth parameters of mung bean plants (root length, shoot length, root fresh and dry weight, shoot fresh and dry weight and No. of leaves per plant) were significantly increased at the treatments cytokinins at the concentration 100 ppm and β-sitosterol at the concentration 10^{-5} M. Similarly, the photosynthetic pigments (chlorophyll a, chlorophyll b, carotonoids and total pigments) as well as the defense enzymes (peroxidase, polyphenoloxidase and catalase) activity and the content of total phenols were significantly increased by the same treatments. Also, the yield parameters (No. of pods per plant, weight of pods per plant, No. of seeds per plant and weight of seeds per plant) were significantly increased in mung bean plants non-infected and infected with *Fusarium oxysporum* treated with cytokinins at the concentration 100 ppm or β-sitosterol at the concentration 10^{-5} M. **Conclusion:** It is evident from the above results that, the resistance of mung bean plant to the used pathogenic fungi (*Fusarium oxyspurm*) was more or less improved by priming the seeds in kinetin and β-sitosterol specially in response to 100 ppm kinetin and 10^{-5} M β-sitosterol, these plant growth regulators could be used, as safe compounds to improve the resistance of mung bean plant to fungal pathogens.

Key words: Mung bean, *Vigna radiate*, *Fusarium oxysporum*, cytokinins, β-sitosterol, performance, productivity, resistance, photosynthetic pigments, total phenols, defense enzymes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The mung bean (*Vigna radiata*) is a member of the legume family (Fabaceae). This family is a wide spread family as it occupies the third largest family of flowering plants, with approximately 650 genera and nearly 20,000 species[1]. Mung bean has many local names "mung bean, mash, golden gram or green gram". The species ranges from large tropical canopy trees to small herbs found in temperate zones, humid tropics, arid zones, high lands, savannas and low lands[2].

Fusarium oxysporum Schlecht. causes vascular wilt diseases in a wide variety of economically important crops[3]. Control of plant diseases still relies mainly on the use of synthetic fungicides, but environmental and health concerns and the development of fungicide-resistant pathogens have stimulated the search for alternative control strategies[4,5]. One approach involves the activation of natural plant defenses to protect crops from losses caused by plant diseases[6]. Plant growth hormones, auxin (Indole-3-acetic acid) and cytokinin (kinetin), suppress *H. maydis* in culture media and in a detached root assay. Kinetin and even more auxin caused significant suppression of fungus spore germination[7].

Cytokinins are phytohormones derived from adenine and are involved in the regulation of root and shoot growth and leaf longevity. Evidence suggests that CK is involved in the regulation of plant defense responses against some pathogens[8]. Moreover, sterols play an important role in plant development including cell expansion, vascular differentiation, etiolation and reproductive development[9,10]. Wheat and rice grains soaked in sterols gave the highest values of root length, shoot length, dry weight of shoot and root and also in germination percentage[11].

The present study aimed at investigating the efficacy of the growth regulators cytokinins and β-sitosterol in controlling the wilt disease of mung bean caused by *Fusarium oxysporum* and their capability to improve the fitness and yield quantity of the plant.

MATERIALS AND METHODS

Isolation, purification and identification of *Fusarium oxysporum*: Diseased mung bean plants showing different degrees of wilt disease were collected from different regions of Dakahlia governorate, (Mansoura, Aga, Sinebellawen and Sherbeen). The affected roots were washed and cut into small portions, immersed in sodium hypochlorite (5% chlorine) for 1 min, washed with distilled water and finally dried between two sterilized filter papers. Then they were directly placed into petri dishes containing Potato Dextrose Agar (PDA) medium and incubated at 28°C for 3-5 days. All the isolated fungi were purified using single spore or the hyphal tip techniques suggested by Dhingra and Sinclair[12]. The purified fungi were identified according to their morphological features according to Booth[13]. Stock cultures were maintained on PDA plants and kept in a refrigerator at 5-10°C and were sub-cultured on fresh medium every 6-8 weeks.

Pathogenicity test of *Fusarium oxysporum*: Earthen ware pots (20 cm in diameter) were sterilized by immersing them in 5% formalin solution for 15 min and covered overnight with plastic sheets, then left to dry in the open air. Soil sterilization was carried out by autoclaving. Susceptible mung bean (*Vigna radiate* L., Wilczek) seeds obtained from Horticulture Research Institute, ARC, Giza were used in this experiment. Mung bean seeds were surface sterilized with 1% sodium hypochlorite for 1 min. Then, they were washed in several changes of sterilized water and left to dry. The surface sterilized seeds were sown in sterilized soil and irrigated with water for 30 days. Bottles containing sand-barley medium were autoclaved and then inoculated with the fungal isolate. After incubation at 28°C for 2 weeks, the fungus was thoroughly mixed with sterilized light loam soil at the rate of 1 g inoculum per 100 g soil. Inoculated pots were watered regularly for a week before planting. Pots used for control were filled with the same soil and mixed only with the same rate of fungus free-sand barley medium and treated in the same way[14]. The transplants were transferred into sterilized infested soil and planted. Other transplants were planted in sterilized uninfested soil. After 30 days from cultivation of transplants the disease incidence was recorded as follows:

$$\text{Disease incidence} = \frac{\text{No. of wilting plants}}{\text{Total No. of plants}} \times 100$$

Effect of cytokinins and β-sitosterol on the linear growth of *Fusarium oxysporum in vitro*: The appropriate amount of cytokinins or β-sitosterol were prepared by mixing it with 50 mL PDA medium in each flask just before solidification to give end concentrations of 200, 100, 50 and 25 ppm of cytokinins and 10^{-1}, 10^{-3}, 10^{-5} and 10^{-7} M of β-sitosterol. Twenty milliliters of each PDA medium was poured in each petri dish and then they were inoculated with 6 mm discs of fungal growth and incubated at 25±2°C. Three replicates were used per each treatment. Petri dishes having no treatments were served as control treatment.

Greenhouse experiment: From the preliminary experiment the concentrations 100 and 50 ppm for kinetin and 10^{-3} and 10^{-5} M for β-sitosterol were selected to be used in field experiment. Two sets of similar pots filled with equal amounts of garden soil (prepared by mixing clay to sand, 2:1, v/v) were prepared.

A homogenously-sized lot of *Vigna radiata* L., Wilczek. (mung bean) seeds were selected then surface sterilization was carried out by soaking the seeds in 0.01% $HgCl_2$ solution for 3 min. The seeds were washed thoroughly with tap water. For each set of pots the seeds were divided into five equal groups, soaked for 2 h as follow; the first soaked in water whereas the other four groups were soaked separately in the first kinetin concentration (100 ppm), second kinetin concentration (50 ppm), first β-sitosterol concentration (10^{-3} M) and second β-sitosterol concentration (10^{-5} M) respectively.

The pots of one set was inoculated with *Fusarium oxysporum*, while the other set not infected. The inoculum was prepared by growing it in glass bottles containing sterilized sorghum grain medium (100 g of sorghum seeds mixed with sand at a ratio of 2:1 (v/v) and moisten with water, transferred to conical flasks and autoclaved at 121°C for 20 min), then incubated at 25±2°C for 15 days. Soil infestation was achieved by mixing the inoculums of *Fusarium oxysporum* fungus at a rate of 4% (w/w). To make the pots ready for seeding, they were irrigated every 2 days for a period of a week to insure fungi adaptation. Six healthy-looking mung bean seeds were sown in each pot. Five pots were used as replicates for each treatments. All seeds of *Vigna radiate* were cultivated at 25th of June, 2014 and the pots were kept in the greenhouse under a normal day per night conditions and irrigated as usual practice with equal amounts of tap water when required. At 1st of September, 2014 samples from treated and untreated, infected and non-infected plants in the fruiting stage were collected for different estimations.

Estimation of photosynthetic pigments: The plant photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined at different stages of plant growth using the spectrophotometric method as recommended by Arnon[15] for chlorophylls and Horvath *et al.*[16] for carotenoids as adopted by Kissimon[17]. A known fresh weight of plant leaves was cut in an ice cold porcelain mortar, some quartz sand was added and a little amount of Na_2CO_3 powder were also to reduce acidity. The leaves were ground with 80% acetone for 5 min. After grinding, the extract was transferred to a centrifuge tube and the volume was completed to 8 mL by acetone. Centrifugation at 1000 rpm for 3 min, the color was measured immediately against a blank of pure 80% aqueous acetone at 3 wavelengths of 480, 644 and 663 nm using spectrophotometer, model (21 D). Taking into consideration the dilutions made, the concentration of the pigment fractions were calculated as μg mL^{-1} using the following equations:

$$\text{Chlorophyll a} = 10.3\ E663 - 0.918\ E644 = \mu g\ mL^{-1}$$
$$\text{Chlorophyll b} = 19.7\ E644 - 3.87\ E663 = \mu g\ mL^{-1}$$
$$\text{Carotenoids} = 5.02\ E480 = \mu g\ mL^{-1}$$

Then, the fractions were calculated as μg g^{-1} dry weight of the differently treated plant leaves.

Total phenols: Fresh leaves of mung bean plants were collected to determine their contents of the total phenols using Foline-ciocalteau reagent[18]. Samples (2 g per each) were homogenized in 80% ethanol, centrifuged at 10000 rpm for 15 min and the supernatant were collected. The obtained residues were re-extracted twice in 80% ethanol and the three collected supernatant was placed into evaporating dishes at room temperature until complete dryness. The residue was dissolved in 5 mL distilled water and 100 μL of the extract was re-diluted in 3 mL of distilled water followed by adding 0.5 mL of Foline-ciocalteau reagent. Three minutes, later 2 mL of 20% sodium carbonate was added and the mixture was vortexed thoroughly and left for 1 h. The developed color was measured photometrically at 650 nm length and catechol was used for calibration. The final results were expressed as milligram (mg) catechol per 100 g fresh weight.

Assay of defense related enzymes activity

Enzyme extraction: Mung bean leaves were collected 72 h after each treatment and directly immersed in liquid nitrogen, lyophilized and then milled to a fine powder with a mortar and pestle. The powder (0.1 g) was extracted using 5 mL sodium phosphate buffer (0.05 M, pH 6.5) for 2 h at 4°C and then centrifuged at 20,000xg for 15 min at 4°C. After centrifugation, the supernatant was saved. The supernatant was designated as the crude leaf extract and its protein content was determined in comparison with a Bovine Serum Albumin (BSA) standard[19].

Peroxidase activity (POD): Peroxidase activity was estimated following the method of Mahadevan and Sridhar[20]. Five milliliters of freshly prepared pyrogallol reagent (prepared by mixing 10 mL of 0.5 M pyrogallol solution and 12.5 mL of 0.66 M phosphate buffer and the volume made to 100 mL

with distilled water) and 1.5 mL of the enzyme extract were mixed in a spectrophotometer tube and the mixture was immediately adjusted to zero absorbance of a spectrophotometer. About 0.5 mL of 1% H_2O_2 solution was added to it and the content was mixed by inverting the tube. The reaction was initiated by the addition of H_2O_2. Enzyme activity was recorded as the change in absorbance per minute ($\Delta A\ min^{-1}$) at 430 nm immediately after the addition of substrate. Similarly, control of non-enzymatic oxidation was maintained by heating at 100°C, where the activity was always measured zero indicating its complete inactivation by the heat treatment.

Polyphenol oxidase enzyme activity (PPO): Polyphenol oxidase activity was measured by the method of Sadasivam and Manickam[21]. Two milliliter of enzyme extract and 3 mL of distilled water were mixed together in a spectrophotometer tube and adjusted to zero absorbance of a spectrophotometer. One milliliter of catechol solution (0.4 mg mL^{-1}) was added to the above mixture and the reactants were quickly mixed. The enzyme activity was measured as the change in absorbance per minute ($\Delta A\ min^{-1}$) at 490 nm immediately after the addition of catechol solution which initiated the reaction. Control in similar manner was maintained by heating at 100°C which always showed zero absorbance.

Catalase enzyme activity: The estimation of the enzyme was carried out according to Devi[22]. One milliliter of the extract was placed in a test tube and 2 mL of 0.1 M H_2O_2 and 3 mL of the phosphate buffer were added. One milliliter of 0.7 N H_2SO_4 (or 10 mL of 2%) was added, to stop the reaction after 5 min. Incubation for 5 min at 27°C and titration the residual H_2O_2 against 0.01 N $KMnO_4$ (taken in the burette). The end point recorded when the pink colour appears and persists for 30 sec. Titration of a blank takes place (1 mL of enzyme extract and 2 mL of H_2O_2 were added to 1 mL of 0.7 N H_2SO_4 immediately and 3 mL of the phosphate buffer) and the titration value recorded. The amount of H_2O_2 destroyed by catalase is calculated by the equation:

$$\text{Catalse activity} = \frac{25 \times 0.85}{2} \times V/W$$

Where:
W = Weight of material used
V = Volume of $KMnO_4$ utilized (Blank-sample value)

The catalase activity presented as enzyme units per gram leaf material. One unit of catalase is defined as that amount of enzyme, which breaks down 1 mmol of $H_2O_2\ min^{-1}$.

RESULTS

Pathogenicity test: The results demonstrated that all isolates were pathogenic and produced typical symptoms of wilt disease *Fusarium oxysporum* isolated from Aga (F_1) recorded the highest values of disease incidence (50%) (Table 1). So this isolate was considered to be the most aggressive one and used for further studies.

Effect of cytokinins and β-sitosterol on the linear growth of *Fusarium oxisporum in vitro*: Table 2 showed that cytokinins at concentrations of 25, 50, 100 and 200 ppm and β-sitosterol at 10^{-1}, 10^{-3}, $10^{-5}$and 10^{-7} M, slightly reduced the linear growth of *F. oxysporum*, isolated from mung bean roots. It was also noticed that the reduction in the linear growth was correlated to the increase in the concentration of the tested growth regulator.

Table 1: Pathogenicity test for different isolates of *F. oxysporum*

Isolates	Location	Disease incidence* (%)
F1	Aga	50
F2	Sherbeen	46
F3	Sinebellawen	42
F4	Mansoura	40

*Each value represent the mean values of 3 replicates

Table 2: Effect of different concentrations of cytokinins and β-sitosterol growth regulators on the linear growth of *Fusarium oxyspom*

	Diameter of fungal growth (cm)	
Treatments	Control	9.00[a]
Cytokinins	25 ppm	8.33[ab]
	50 ppm	7.57[a-c]
	100 ppm	7.06[bc]
	200 ppm	5.86[cd]
β-sitosterol	10^{-7} M	8.69[ab]
	10^{-5} M	7.49[a-c]
	10^{-3} M	6.49[cd]
	10^{-1} M	5.16[d]

Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p = 0.05

Table 3: Effect of cytokinins and β-sitosterol on wilt disease of mung bean plant non-infected and infected with *Fusarium oxysporum*

	Treatments	Wilt disease incidence (%)
Control (non-infected)	Water	27.69[a]
	Cytokinins (50 ppm)	23.69[b]
	Cytokinins (100 ppm)	8.31[d]
	β-sitosterol (10^{-3} M)	20.61[c]
	β-sitosterol (10^{-5} M)	0.00[e]
Fusarium oxysporum	Water	50.15[a]
	Cytokinins (50 ppm)	46.15[b]
	Cytokinins (100 ppm)	26.77[d]
	β-sitosterol (10^{-3} M)	30.77[c]
	β-sitosterol (10^{-5} M)	11.38[e]

Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p = 0.05

Table 4: Effect of cytokinins and β-sitosterol on the growth parameters of mung bean plants non-infected and infected with *F. oxysporum*

	Treatments	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)	No. of leaves per plant
Control (non-infected)	Water	5.78^{e*}	45.33^{d}	19.00^{d}	1.65^{e}	33^{e}	5.49^{e}	11.67^{e}
	Cytokinins (50 ppm)	6.20^{d}	48.67^{c}	23.00^{c}	1.99^{d}	46^{d}	7.61^{d}	13.33^{d}
	Cytokinins (100 ppm)	6.84^{b}	53.67^{b}	25.33^{b}	2.20^{b}	66^{b}	10.97^{b}	16.67^{b}
	β-sitosterol (10^{-3} M)	6.58^{c}	51.67^{b}	23.33^{c}	2.04^{c}	55^{c}	9.12^{c}	14.67^{c}
	β-sitosterol (10^{-5} M)	7.53^{a}	59.00^{a}	28.33^{a}	3.43^{a}	84^{a}	14.04^{a}	21.67^{a}
Fusarium oxysporum	Water	3.93^{e}	30.67^{e}	11.33^{e}	0.98^{e}	27.33^{e}	4.55^{e}	8.33^{e}
	Cytokinins (50 ppm)	4.32^{d}	33.67^{d}	13.00^{d}	1.14^{d}	32.67^{d}	5.46^{d}	10.67^{d}
	Cytokinins (100 ppm)	5.08^{b}	39.67^{b}	16.00^{b}	1.44^{b}	34.67^{b}	7.13^{b}	12.33^{b}
	β-sitosterol (10^{-3} M)	4.59^{c}	36.00^{c}	14.67^{c}	1.27^{c}	34.00^{c}	6.49^{c}	11.33^{c}
	β-sitosterol (10^{-5} M)	5.32^{a}	41.67^{a}	18.67^{a}	1.60^{a}	35.00^{a}	8.07^{a}	17.67^{a}

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p = 0.05

Table 5: Effect of cytokinins and β-sitosterol on the content of the photosynthetic pigments (mg g^{-1} fresh weight) in mung bean plants non-infected and infected with *F. oxysporum*

	Treatments	Chlorophyll a	Chlorophyll b	Total chlorophyll	Chlorophyll a/ Chlorophyll b ratio	Carotenoid	Total pigments
Control (non-infected)	Water	4.03^{d}	3.31^{d}	7.34^{d}	1.22^{d}	1.75^{c}	9.09^{e}
	Cytokinins (50 ppm)	5.57^{c}	3.49^{c}	9.06^{c}	1.60^{c}	1.79^{c}	10.85^{d}
	Cytokinins (100 ppm)	7.77^{b}	4.89^{b}	12.66^{b}	1.59^{c}	2.01^{b}	14.67^{b}
	β-sitosterol (10^{-3} M)	5.88^{c}	3.50^{c}	9.38^{c}	1.68^{b}	1.86^{c}	11.24^{c}
	β-sitosterol (10^{-5} M)	9.70^{a}	5.46^{a}	15.16^{a}	1.78^{a}	2.33^{a}	17.49^{a}
Fusarium oxysporum	Water	2.48^{d}	2.38^{d}	4.86^{d}	1.04^{d}	0.96^{d}	5.82^{e}
	Cytokinins (50 ppm)	3.58^{c}	2.46^{c}	6.04^{c}	1.46^{c}	1.51^{c}	7.55^{d}
	Cytokinins (100 ppm)	4.82^{b}	3.08^{b}	7.90^{b}	1.56^{b}	1.81^{b}	9.71^{b}
	β-sitosterol (10^{-3} M)	3.71^{c}	2.73^{bc}	6.44^{c}	1.36^{cd}	1.71^{b}	8.15^{c}
	β-sitosterol (10^{-5} M)	6.63^{a}	3.30^{a}	9.93^{a}	2.01^{a}	2.09^{a}	12.02^{a}

Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p = 0.05

Table 6: Effect of cytokinins and β-sitosterol on the peroxidase, polyphenoloxidase, catalase activity and the content of the total phenols in mung bean plants non-infected and infected with *F. oxysporum*

	Treatments	Peroxidase	Polyphenol oxidase	Total phenol	Catalase (mg catechol per 100 g fresh weight)
Control (non-infected)	Water	0.202^{c*}	0.012^{e}	0.113^{e}	149^{e}
	Cytokinins (50 ppm)	0.233^{c}	0.014^{d}	0.191^{d}	192^{d}
	Cytokinins (100 ppm)	0.459^{ab}	0.025^{b}	0.283^{b}	306^{b}
	β-sitosterol (10^{-3} M)	0.356^{bc}	0.021^{c}	0.240^{c}	213^{c}
	β-sitosterol (10^{-5} M)	0.570^{a}	0.045^{a}	0.93^{a}	353.3^{a}
Fusarium oxysporum	Water	0.084^{d}	0.018^{d}	0.001^{e}	129^{e}
	Cytokinins (50 ppm)	0.283^{c}	0.020^{c}	0.007^{d}	161^{d}
	Cytokinins (100 ppm)	0.352^{b}	0.027^{b}	0.205^{b}	219.7^{b}
	β-sitosterol (10^{-3} M)	0.285^{c}	0.021^{c}	0.134^{c}	171^{c}
	β-sitosterol (10^{-5} M)	0.394^{a}	0.030^{a}	0.460^{a}	389.7^{a}

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p = 0.05

Greenhouse experiment: The recorded data Table 3 cleared that both cytokinins and β-sitosterol significantly reduced the percentage of the incidence of wilt disease of mung bean infected with *F. oxysporum*. Precisely, the β-sitosterol at 10^{-5} was the most effective treatment in reducing the incidence of wilt (0.00).

The results represented in Table 4 showed that the treatment of uninfected (control) and infected with *F. oxysporum* mung bean plants with citokinins or β-sitosterol significantly increased all the growth parameters (root length, shoot length, root fresh and dry weight, shoot fresh and dry weight and No. of leaves per plant) compared to not treated (control) plants. The increase was more apparent in case of cytokinins at the concentration 100 ppm and β-sitosterol at the concentration 10^{-5} M.

Data represented in Table 5 showed that all the determined photosynthetic pigments (chlorophyll a, chlorophyll b, carotonoids and total pigments) were increased in mung bean plants (non-infected and infected with *Fusarium oxysporum*) treated with cytokinins or β-sitosterol. It was obvious that the increase was more significant in case of cytokinins 100 ppm and β-sitosterol 10^{-5} M.

The impact of growth regulator hormones on the peroxidase, polyphenoloxidase, catalase activity and the

Table 7: Effect of cytokinins and β-sitosterol on the yield attributes of mung bean plants non infected and infected with *F. oxysporum*

	Treatments	No. of pods per plant	Weight of pods per plant	No. of seeds per plant	Weight of seeds per plant
Control (non-infected)	Control	8.7^{c*}	63.3^{e}	30.3^{d}	52^{e}
	Cytokinins (50 ppm)	10.7^{c}	95^{d}	37.7^{c}	78^{d}
	Cytokinins (100 ppm)	16.3^{b}	156.7^{b}	58^{b}	129^{b}
	β-sitosterol (10^{-3} M)	15.7^{b}	125.3^{c}	55.3^{b}	103^{c}
	β-sitosterol (10^{-5} M)	19.3^{a}	188^{a}	69^{a}	155^{a}
Fusarium oxysporum	Control	8.7^{c}	62.7^{c}	30.3^{d}	51.7^{e}
	Cytokinins (50 ppm)	10.3^{b}	67.7^{c}	37^{cd}	56^{d}
	Cytokinins (100 ppm)	13.7^{a}	108.7^{b}	48.7^{ab}	89^{b}
	β-sitosterol (10^{-3} M)	11.7^{b}	104.7^{b}	41^{bc}	83.3^{c}
	β-sitosterol (10^{-5} M)	14.3^{a}	141^{a}	51^{a}	116^{a}

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p = 0.05

content of total phenols in mung bean plants non-infected and infected with *F. oxysporum* (Table 6) revealed that β-sitosterol at the concentration 10^{-5} M significantly increased the peroxidase, polyphenol oxidase and catalase activities as well as total phenols (0.570, 0.045, 0.93 and 353.3, respectively) in non-infected plants and (0.394, 0.030, 0.460 and 389.7, respectively) in infected plants.

Fortunately, the treatment of both non-infected and mung bean plant infected with *Fusarium oxysporum* with cytokinins (50 and 100 ppm) and β-sitosterol (10^{-5} and 10^{-3} M) significantly increased the yield parameters (Table 7). The increase in the yield parameters (No. of pods per plant, weight of pods per plant, No. of seeds per plant and weight of seeds per plant) was more obvious in case of β-sitosterol 10^{-5} M (19.3, 188, 69 and 155, respectively) in the non-infected plants and (14.3, 141, 51 and 116, respectively) in the infected plants.

DISCUSSION

Control of plant diseases still relies mainly on the use of synthetic fungicides, but environmental and health concerns and the development of fungicide-resistant pathogens, have stimulated the search for alternative control strategies[4,5]. One approach involves the activation of natural plant defenses to protect crops from losses caused by plant diseases[6].

Acquired resistance by using abiotic-agents as inducers seems to be one of alternatives to substitute for or at least to decrease the use of fungicides in plant disease control. Excessive and improper use of pesticides including fungicides presents a menace to the health of human, animal and environment[23]. In the present study, it was planning to investigate the possibility of minimizing the infection with wilt disease of mung bean using the growth regulators, cytokinins and β-sitosterolas resistance inducer.

The obtained data revealed that both cytokinins and β-sitosterol not only had antifungal activity against *Fusarium oxysporum* and inhibited its linear growth on PDA plates but also significantly reduced the percentage of the incidence of wilt disease of mung bean infected with *F. oxysporum* in the greenhouse experiment.

Some investigations indicated that Naphthalene Acetic Acid (NAA) is a potential antifungal agent[24-26]. Auxins strongly inhibited mycelium growth, sporulation and spore germination of *Fusarium culmorum in vitro*[27]. The NAA, Indole Acetic Acid (IAA), 2,4-diphenol acetic acid (2,4,D) and abscisic acid (ABA) were exogenously applied to control *Alternaria solani* caused early blight of potato. Auxins such as IAA, naphthalene acetic acid ethyl ester and N-metatotylphthalamic acid reduced Botrytis blight of cut rose flowers[28].

The obtained data revealed that both citokinins and β-sitosterol significantly increased all the growth parameters (root length, shoot length, root fresh and dry weight, shoot fresh and dry weight and No. of leaves per plant) of mung bean plant, non-infected and infected with *Fusarium oxysporum* compared to untreated (control) plants. This results are in agreement with that of Zhang and Schmidt[29] who reported that application of Hormone Containing Products (HCP) on Kentucky blue grass, improved leaf water status and enhanced root and shoot growth. Furthermore, kinetin-treated wheat plants subjected to salinity stress, kinetin ameliorated the deleterious effect of stress[30].

Data obtained in the present study indicated that treatments with hormonal elicitors (cytokinins or β-sitosterol) markedly affected the efficiency of photosynthetic pigments (chlorophyll a, chlorophyll b, carotonoids and total pigments) in non-infected and infected mung bean leaves with a better potential for resistance. According that, the decrease in photophosphorylation rate, which usually occurring after an infection[31] can be compensated by an increase in efficiency of the photosynthetic apparatus. Also, high chlorophyll content in cytokinins and β-sitosterol treated plants could be attributed to their stimulatory effect on rubiso activity[32].

Data of the present study revealed that the treatment of both non-infected and mung bean plant infected with

Fusarium oxysporum in addition to cytokinins (50 and 100 ppm) and β-sitosterol (10^{-5} and 10^{-3} M) significantly increased the yield parameters (No. of pods per plant, weight of pods per plant, No. of seeds per plant and weight of seeds per plant). The increase in yield parameters may be attributed to the promotion of cell division, nutrient mobilization and leaf longevity by growth hormones[33,34]. Also, Kurakawa *et al.*[35] and Ashikari *et al.*[36] reported that cytokinins can also increase grain yield, for example by activating inflorescence meristem activity in rice (*Oryza sativa*). Interestingly, sitosterol also exhibit bacteriostatic or bactericidal activity against a broad range of Gram-positive and Gram-negative organisms, as well as *Candida albicans*[37].

This results indicated that, the activity of the resistance related enzymes (peroxidase, polyphenoloxidase and catalase) as well as the total phenol content were significantly increased by the treatment of mung bean plant non-infected and infected with *F. oxysporum* with β-sitosterol at the concentration 10^{-3} M. These results were in agreement with that of Choi *et al.*[38], which revealed that plant-originated cytokinins augment plant immunity together with Salicylic Acid (SA) signaling. Generation of active oxygen species, i.e., hydrogen peroxidase, superoxide radical and other activated oxygen species in the plant cell wall and in the plasma membrane is often considered to be a defensive oxidative barrier to phytopathogenic fungi[39,40].

CONCLUSION

It is evident from the above results that, the resistance of mung bean plant to the used pathogenic fungi (*Fusarium oxyspurm*) was more or less improved by priming the seeds in kinetin and β-sitosterol specially in response to 100 ppm kinetin and 10^{-5} M β-sitosterol, these plant growth regulators could be used, as safe compounds to improve the resistance of mung bean plant to fungal pathogens. The mechanism of the used growth regulators in stimulating the plant defense against fungal pathogens may be due to increasing the determined photosynthetic pigments, total phenols and antioxidant enzymes.

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