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Suppression of *Cephalosporium maydis* by the resistance inducer beta-sitosterol

Ibrahim E. Elshahawy ·
Mohamed S. Abd El-Wahed

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Abstract Late wilt, a vascular disease caused by the fungus of *Cephalosporium maydis*, is considered one of Egypt's most severe maize threats. The purpose of this study was to investigate the suppressive effect of the resistance inducer beta-sitosterol on *C. maydis*, as well as its involvement in reducing the incidence of late wilt infection under greenhouse and field conditions. In in vitro studies on potato dextrose yeast extract agar (PDYA) and/or potato dextrose yeast extract broth (PDYB) with doses of 50, 100, 150, 200, and 250 ppm, beta-sitosterol significantly reduced colony diameter and spore germination of *C. maydis*. The efficiency of beta-sitosterol increased with concentration, with 250 ppm being the most efficient, reducing colony development by 100% and spore germination by 77.3%. Experiments were conducted in greenhouse and field trials using the split-plot design with three beta-sitosterol 250 ppm application methods (maize grain dipping, maize foliar spraying, and maize grain dipping with foliar spraying) and two maize cultivars (a land race and the cultivar fine seed 1005). In both trials, the combination treatment of maize grain dipping and foliar spraying with beta-sitosterol 250 ppm was most effective. Under greenhouse conditions, beta-sitosterol treatments significantly improved the growth parameters (plant height, plant fresh weight, and plant dry weight) of the two maize cultivars. Under similar conditions, beta-sitosterol significantly increased the activity of protective enzymes (peroxidase, polyphenoloxidase, and chitinase) and the levels of chlorophyll, total phenols, and flavonoids in the two maize cultivars. When compared to the untreated control, beta-sitosterol application reduced the incidence of late wilt disease under greenhouse and field conditions. The ear yield of the two maize cultivars was significantly increased in plots treated with beta-sitosterol 250 ppm in a field trial. The findings showed that beta-sitosterol inhibited *C. maydis* growth in vitro and improved maize plant resistance to late wilt infection in vivo. As a result, this plant resistance inducer could be used to improve the resistance of maize cultivars to late wilt disease.

Keywords Late wilt · *Cephalosporium maydis* · Beta-sitosterol · Maize plants

I. E. Elshahawy (✉)
Plant Pathology Department, National Research Centre, Cairo 12622, Egypt
e-mail: ibrahim_nrc@yahoo.com

M. S. Abd El-Wahed
Department of Botany, National Research Centre, Cairo 12622, Egypt

Introduction

Maiz (*Zea mays* L.) is the world's third-largest cereal crop after wheat and rice, and it is grown in a variety of agro-ecolosystems. One of the most important factors in lowering this crop's potential yield is the presence of fungal diseases. They reduce both the quantity and type of grain produced, and they may increase cost of cultivation. Late wilt, also known as black bundle disease, is a vascular wilt disease of maize caused by the fungus *Cephalosporium maydis* Samra, Sabet, and Hingorani (Samra et al., 1963).

The fungus *Cephalosporium maydis* was first identified in Egypt in 1960 as a cause of vascular wilt in maize, and it is now considered endemic throughout Egypt (Samra et al., 1963). It has also been discovered in maize from other countries (Payak et al., 1970; García-Carneros et al., 2011; Molinero-Ruiz et al., 2011; Drori et al., 2012). Serious economic losses as a result of late wilt have been reported in Egypt, where 100% incidence occurs in some fields, and in India, where incidence is as high as 70% and economic losses are as high as 51% (Elshahawy & El-Sayed, 2018; Labib et al., 1975; Payak et al., 1970). The fungus reproduces asexually, and there is no perfect stage (Saleh, A.A.,& Leslie, J.F., 2004). The pathogen survives as sclerotia on maize debris and infects maize seedlings through the roots or mesocotyl (El-Assiuty et al., 1999). At first, it grows on roots epiphytically, producing short, thick-walled hyphae with swollen cells similar to *Gaeumannomyces hyphopodia* (Saleh, A.A.,& Leslie, J.F., 2004). Penetration can occur anywhere on the root system or mesocotyl (except at the root tips), but it is most common where lateral roots originate or where root elongation occurs (Sabet et al., 1970; Sadik, 1973). As epidermal cells degrade, the fungus penetrates the xylem and grows intra- and intercellularly (Zeller et al., 2002). Root damage predisposes plants to the disease, and insect or nematode damage creates new entry points (Payak et al., 1970; Singh & Siradhana, 1988). During the first five weeks after maize germination, the fungus spreads slowly before rapidly spreading upward throughout the plant. It is distributed throughout the stalk until flowering (anthesis at 9–10 weeks), at which point many vessels are blocked by hyphae and a dark gum-like substance (Abd El-Rahim et al., 1998; Sabet et al., 1970). The root tips of infected maize plants are stained red during the early stages of infection, but above ground parts are generally symptomless until tasseling, when a rapid wilting of lower leaves progresses upward (Sabet et al., 1970). The leaves appear streaked before rolling inward and appearing scorched while retaining some of their green color as the tissue between the veins turns dull green and then chlorotic. On the stalk's basal internodes, yellow to reddish brown streaks appear. Wilting can occur suddenly, allowing non-infected ("escapes") or resistant plants to be distinguished. Stalks are dry and hollow, with macerated pith that ranges from dark yellow to brownish and vascular bundles that are brownish-black. Infected stalks' lower parts dry out, shrink, and become hollow. Secondary invader infections such as *Cephalosporium acremonium*, *Sclerotium bataticola*, *Fusarium verticillioides*, and various bacterial rots are frequently associated with late wilt, resulting in a "stalk rot complex." (Samra et al., 1963). Fewer ears are produced, and the kernels that do form are underdeveloped and may contain pathogens (Ortiz-Bustos et al., 2016).

The most cost-effective way to manage late wilt is to develop genetically resistant maize lines. This method is environmentally friendly, efficient, and cost effective. Effective late wilt disease risk management, particularly the avoidance of sensitive maize cultivars, may contribute to this positive trend. Nonetheless, highly aggressive *C. maydis* isolates discovered in recent years (García-Carneros et al., 2011; Ortiz-Bustos et al., 2016) may pose a threat to previously resistant maize cultivars, which can lose immunity after extensive cultivation over long periods of time. As a result of this worrying situation, researchers are looking for alternative methods (Abdel-Monem et al., 2020; Darwesh et al., 2019; Darwesh & Elshahawy, 2021; Elshahawy et al., 2021; Elshahawy & Saied, 2021). There have been several attempts to use biological methods to manage the late wilt infection (El-Assiuty et al., 1991; El-Mehalowy et al., 2004; Elshahawy & El-Sayed, 2018). Synthetic fungicides have long been used to control fungal diseases on plants. However, growing resistance to such fungicides, as well as environmental side effects have necessitated the development of environmentally friendly fungicides (Leonard et al., 2007). Plants produce a plethora of optional metabolites that should protect them from microbial diseases (Dixon, 2001). Some of these natural products have been shown to act as botanical fungicides and may be useful in the control of agricultural diseases (Dayan et al., 2009). Botanical fungicides are in high demand due to their abundance, selectivity, ease of degradation, and nontoxicity to mammals (Leonard et al., 2007). Some botanical pesticides, such as curcuminoids, cinnamaldehyde, neem, and pyrethins, are used commercially to control phytopathogenic fungi (Copping & Duke, 2007; Leonard et al., 2007; Yoon et al., 2013).

Phytosterols, which resemble cholesterol in structure, are a subset of steroids, a large family of bioorganic compounds found in plants, animals, and fungi. These chemicals have a long history of use as food or pharmaceutical ingredients, and they are widely regarded as safe, with no negative side effects (Saeidnia et al., 2014). One of these phytosterols that has been studied in vitro for its antifungal and antibacterial properties is beta-sitosterol (Campos et al., 2014; Choi et al., 2017). In vivo, beta-sitosterol is commonly used to mitigate the negative effects of

various biotic and abiotic stresses, such as drought, salinity, heat, cold, ultraviolet-B (UV-B) radiation, and pathogen attack, in order to improve crop performance under current growth conditions (Divi & Krishna, 2009; Elkeilsh et al., 2019; Shahzad et al., 2021; Yenjit et al., 2010). There have been no published studies on the antifungal activity of beta-sitosterol compounds against *C. maydis*. Therefore, the objectives of this study are to: (1) evaluate the antifungal activity of beta-sitosterol against *C. maydis* in vitro, (2) evaluate the effect of beta-sitosterol application on late wilt disease incidence, plant growth, and the induction of defense enzymes in maize plants grown under greenhouse conditions, and (3) evaluate the effect of beta-sitosterol application on late wilt disease incidence and grains yield in maize plants grown under field conditions.

Materials and methods

Chemicals

Beta-sitosterol $C_{29}H_{50}O$ (Synonym(s): 22, 23-Dihydrostigmasterol, beta-Sitosterol, 5-Stigmasten-3 β-ol,α-Dihydrofucosterol, 24α-Ethylcholesterol) compound was purchased from Sigma-Aldrich (C83–46-5,MW 414.71). PDA (Potato Dextrose Agar), YE (Yeast Extract) and PDB (Potato Dextrose Broth) were from Merck KGaA, 64,271 Darmstadt Germany. DMSO (Dimethyl sulfoxide) was purchased from Sigma-Aldrich (C67–68-5, MW 78.13).

Maize cultivars

The susceptible cultivar Land race was provided by the Crops Department, Agricultural and Biology Institute, National Research Centre, and the moderately resistant cultivar Fine seed 1005 by the Fine Seeds International company, Giza, Egypt. Maize grains were thoroughly cleaned (to remove any remaining pesticide coatings) by rinsing them with water and immersing them in a large amount of sterilized distilled water for 16 h while stirring constantly. Surface sterilization of maize grains was accomplished by immersing them in a 2% NaOCl solution for 2 min, followed by a 2 min soak in 70% ethanol. The seeds were then rinsed four times with sterilized water.

Late wilt pathogen

In this study, Cm3, a virulent isolate of *Cephalosporium maydis*, was chosen and used. Cm3 was previously isolated from wilting maize plants collected during the 2018 growing season in a maize field in Gharbia Governorate, Egypt (Elshahawy & El-Sayed, 2018). As previously described, the pathogen was isolated from infected plants (Zeller et al., 2002). Symptomatic plant internodes were sterilized in 2% sodium hypochlorite and split with a sterile knife; a small piece of each internode's discolored vascular bundle was placed on sterilized Petri plates containing autoclaved sterile PDYA (39 g PDA, 2 g yeast extract and 1000 ml distilled water). *C. maydis* cultures were propagated from single conidia and identified based on cultural and microscopic characteristics (production of «heads» of hyaline, non-septate conidia from simple phialides) (Samra et al., 1963). According to Koch's postulates, the pathogenicity of this isolate was confirmed in this study using a susceptible "land race" cultivar, and the pathogen was re- isolated and characterized by colony morphology and microscopic traits (Fig. 1). The isolate was grown in complete darkness on PDYA at 28 ± 2 °C. A 1000-ml Erlenmeyer flask containing 200 g of wet autoclaved sterile sorghum grains was inoculated with 10 colony agar discs (5-mm diameter each) taken from the margins of a 7-day-old colony of *C. maydis* to make inocula. Cultures were grown in an incubator at 28 ± 2 °C in the dark for four weeks. The inoculum was then harvested and homogenized.

Fig. 1 Pathogenicity test of *Cephalosporum maydis* (Cm3 isolate) on Land races cv. of maize plants under greenhouse conditions. Severe disease symptoms appeared in maize plants infected with Cm3 isolate (Right), while in non-infected plants (Left) the plants developed normally, photographed 65 days after sowing

Laboratory experiments

Antifungal activity of beta-sitosterol using agar plate assays

On agar plates, an in vitro evaluation of the beta-sitosterol compound on the radial mycelial growth of *C. maydis* was performed (Ali et al., 2016; Degani et al., 2014). There were five different beta-sitosterol concentrations tested (50, 100, 150, 200 and 250 ppm). By dissolving beta-sitosterol in DMSO, a stock solution of beta-sitosterol was created. The stock's final concentration was 1000 ppm. A 0.22 µm syringe filter (MS®, CA Syringe Filter, Egypt) was used to filter the stock solutions. The antifungal activity test media were created after cooling to 55 °C by adding the appropriate concentration of beta-sitosterol to an autoclaved PDAY. We poured 15 ml of these media with varying beta-sitosterol concentrations into a 9 cm diameter Petri plate. Following solidification, each plate was inoculated with a culture agar disc (5 mm-diameter) cut from the borders of 7-day-old *C. maydis* colonies, including the control (DMSO without beta-sitosterol). Labeled Petri plates were placed in a 28 ± 2 °C dark incubator.. Six replicate plates were made for each treatment (beta-sitosterol at varying rates and the control). We measured the distance across two perpendicular lines from the underside of the Petri plates seven days after inoculation to determine outspread mycelial development. To calculate inhibition (%) in colony diameter of *C. maydis*, we used the following formula: I = C-T/C × 100 I = ((C-T)/C)×100, where C represents colony diameter of *C. maydis* in the control and T represents colony diameter of *C. maydis* in the beta-sitosterol treatment. Each experiment was carried out twice.

Antifungal activity of beta-sitosterol using a spore germination assay

Conidia were harvested from mature *C. maydis* cultures grown on PDYA for seven days at 28 ± 2 °C by rinsing and scraping the agar surface with 1 ml sterile deionized water (Degani et al., 2014). The spores were suspended in a solution of 2% PDBY (about 50 spores µl) containing the desired beta-sitosterol concentrations (50, 100, 150, 200, and 250 ppm). As a control, spores suspended in 2% PDBY but lacking beta-sitosterol were used. Following careful mixing, 0.2 ml of each treatment was pipetted onto sterile glass microscope slides, and the suspension was kept within a rectangle outlined on the slides with liquid paraffin wax. The prepared slides were incubated for 18 h at 28 ± 2 °C in moist Petri dishes. Following that, microscopy was used to perform direct counts to determine the rate of germination. To calculate inhibition (%) in spore germination of *C. maydis*, we used the following formula: I = C-T/C × 100, where C represents spore germination of *C. maydis* in the control and T represents spore germination of *C. maydis* in the beta-sitosterol treatment. Each experiment was carried out twice.

Greenhouse experiments

Experimental unit

During the summer season of 2020, the experiment was carried out in the greenhouse at the Plant Pathology Department, National Research Centre, Giza, Egypt, at an average temperature of 20 °C -30 °C (the minimum) and 32 °C -40 °C (the maximum). The potting soil infestation technique was used as described by El-Shafey et al. (1988). Plastic pots 30 cm in diameter and 35 cm in depth (previously sterilized by soaking in a 7% formaldehyde solution for 10 min and aerating for ten days) were filled with autoclaved clay loam soil, 6 kg/pot. The soil in pots was infested with the previously prepared *C. maydis* inoculum at a rate of 30 g inocula / kg soil, thoroughly mixed to ensure even distribution of fungal propagules, and then irrigated. Pots containing autoclaved sorghum grains free of *C. maydis* inocula served as negative controls. Infested and non-infested potting soils were kept moistened for seven days before sowing. The cultivars used were Land races, a susceptible cultivar, and Fine seed 1005, a relatively resistant cultivar. Five grains of each cultivar were sown in each pot (experimental unit) on 14/6/2020, and plants were thinned to two plants per pot seven days later. Before sowing, 11 g of phosphate rock (P_2O_5) were added per pot, and 5.5 g of ammonium nitrate were added per pot. At 20, 30, and 40 days after sowing, 5.5 g pot^{-1} ammonium nitrate fertilizer was applied, and plants were irrigated as needed (Sadik, 1973).

Beta-sitosterol application

The study was conducted in six replicates using a split-plot design. The first factor was the random assignment of maize cultivars to major plots within blocks. Sub-

plots were created from the main plots (split-plots). The methods for using beta-sitosterol as a second factor were assigned to the sub-plots at random. The main plots were assigned two cultivars: (1) Land races and (2) Fine seed 1005.

In the sub-plots, the treatments were as follows (Table 1): (1) maize grain dipping, (2) maize foliar spraying, (3) maize grain dipping + maize foliar spraying, (4) negative control (non-treated grains sowing in non-infested soil), and (5) positive control (non-treated grains sowing in infested soil). In the laboratory experiments, beta-sitosterol at a concentration of 250 ppm was found to be the most effective treatment. Therefore, it was used under greenhouse conditions. The experiment was repeated twice, each time with the same arrangement. The first experiment was carried out 35 days after planting to look into the effect of beta-sitosterol on maize growth promotion, chlorophyll content, total phenols, flavonoids, and the induction of defense enzymes in maize plants (Experiment I). The second experiment was carried out 90 days after sowing to see how beta-sitosterol affected maize late wilt (Experiment II).

Effects on maize plant promotion

The maize plants were harvested at 35 days of age by mulching the plants from the pots to investigate the effect of beta-sitosterol treatment on plant growth (Experiment I). The fresh weight (g plant^{-1}) and plant height (cm plant^{-1}) of maize plants were measured. The plants were dried at 70 °C until they reached a constant weight, and the dry weight per plant was recorded.

Effects on induction of defense enzymes in maize plants

The effect of beta-sitosterol treatment on defense enzyme activity, such as peroxidase, polyphenoloxidase, and chitinase, was measured 35 days after planting in greenhouse-grown maize plants (Experiment I). Maize leaf samples (g) were homogenized at 0 °C with 0.2 M

Table 1 Methods of beta-sitosterol application under greenhouse and field conditions

Treatment	Description	Method of application
Greenhouse trial		
T1	Negative control	Maize grains were soaked before sown in sterilized distilled water for 3 h. Thereafter, maize grains were sown in pots containing un-inoculated sterilized soil. At 10 and 25 days after sown maize plants were sprayed with sterilized distilled water.
T2	Positive control	Maize grains were soaked before sown in sterilized distilled water for 3 h. Thereafter, maize grains were sown in pots containing sterilized soil artificially infested with *C. maydis*. At 10 and 25 days after sown maize plants were sprayed with sterilized distilled water.
T3	Beta-sitosterol soaking	Maize grains were soaked before sown in 250 ppm beta-sitosterol for 3 h. Thereafter, maize grains were sown in pots containing sterilized soil artificially infested with *C. maydis*.
T4	Beta-sitosterol spraying	Maize grains were sown in pots containing sterilized soil artificially infested with *Cephalosporum maydis*. At 10 and 25 days after sown maize plants were sprayed with 250 ppm beta-sitosterol.
T5	Beta-sitosterol soaking plus spraying	Maize grains were soaked before sown in 250 ppm beta-sitosterol for 3 h. Thereafter, maize grains were sown in pots containing sterilized soil artificially infested with *C. maydis*. At 10 and 25 days after sown maize plants were sprayed with 250 ppm beta-sitosterol.
Field trial		
T1	Untreated control	Maize grains were soaked before sown in sterilized distilled water for 3 h. Thereafter, maize grains were sown in field soil naturally infested with *C. maydis*. At 10 and 25 days after sown maize plants were sprayed with sterilized distilled water.
T2	Beta-sitosterol soaking	Maize grains were soaked before sown in 250 ppm beta-sitosterol for 3 h. Thereafter, maize grains were sown in field soil naturally infested with *C. maydis*.
T3	Beta-sitosterol spraying	Maize grains were sown in field soil naturally infested with *C. mayds*. At 10 and 25 days after sown maize plants were sprayed with 250 ppm beta-sitosterol.
T4	Beta-sitosterol soaking plus spraying	Maize grains were soaked before sown in 250 ppm beta-sitosterol for 3 h. Thereafter, maize grains were sown in field soil naturally infested with *C. maydis*. At 10 and 25 days after sown maize plants were sprayed with 250 ppm beta-sitosterol.

Tris HCl buffer (pH 7.8) containing 14 mM -mercaptoethanol at a rate of 1/3 w/v to extract the enzymes. The supernatants were obtained by filtering out the trash with a clean cloth and centrifuging for 15 min at 3000 rpm. The supernatants were collected and stored in a tube in an ice bath until they were analyzed. A UV spectrophotometer was used to measure the enzyme activity in the supernatant. Using guaiacol as a hydrogen donor, peroxidase activity was determined, and the increase in absorbance at 470 nm/g fresh weight/min was calculated using Lee's technique (Lee, 1973). As described by Bashan et al. (1985), polyphenoloxidase activity was determined by calculating the rate of quinine formation as a result of oxidizing 3,4-dihydroxyphenylalanine (DOPA), and this activity was expressed as an increase in absorbance at 475 nm/g fresh weight/min. Using the Monreal and Reese (1969) technique, chitinase activity was confirmed using colloidal chitin as a substrate and dinitrosalicylic acid (DNS) as a reagent to quantify reducing sugars. At 540 nm, chitinase activity was measured and expressed as mM N-acetyl glucose amine equivalent released/g fresh weight/60 min.

Effects on chlorophyll contents, total phenols and flavonoids in maize plants

Total chlorophyll was measured 35 days after sowing (Experiment I), as described by Königer and Winter (1993). The pigment concentration was determined and expressed in mg g^{-1} of fresh leaf weight. Total phenols and flavonoids were also measured and reported as mg g^{-1} dry weight of leaves by Chandra et al. (2014).

Effects on late wilt incidence

Late wilt disease symptoms usually appear when the plants are about to tassel. Their appearance, on the other hand, can vary from shortly before tasseling to shortly before maturity (Samra et al., 1963). In accordance with this, late wilt disease assessments were performed at regular intervals beginning 60 days after planting and based on wilt symptom manifestations, which included the following: The leaves of infected plants turn pale green, roll inward, and appear thirsty, eventually drying out. The stem's vascular bundles darken to a yellow-brown color as the drying vascular ascends upwards. The percentage of infected plants with late wilt infection was calculated at the end of the trial (90 days after planting, Experiment II) as follows: Late wilt disease (%) = [number of dead plants due to late wilt infection during the growing season / total number of maize plants] × 100.

Field experiment

During the 2020 growing season in Gharbia Governorate, Egypt, the impact of beta-sitosterol application on the incidence of maize late wilt as well as ear yield was investigated in the field. This field (clay soil) site has a history of late wilt disease and is naturally infested with a high *C. maydis* inoculum (Elshahawy & El-Sayed, 2018). In this study, the land race and Fine seed 1005 maize grains were used. The study was conducted in four replicates using a split-plot design. As the first factor, maize cultivars were assigned at random to the main plots within blocks. Sub-plots were created from the main plots (split-plots). The methods for using beta-sitosterol as a second factor were then assigned to the sub-plots at random. Two cultivars were assigned to the main plots: (1) Land race and (2) Fine seed 1005. Table 1 shows the treatments used in the sub-plots: (1) maize grain dipping, (2) maize foliar spraying, (3) maize grain dipping + maize foliar spraying, and (4) untreated control. In field experiments, beta-sitosterol was applied at a concentration of 250 ppm, which proved to be the most effective treatment in the laboratory. Each replication plot had five 6 m long and 0.7 m wide ridges. In each replication plot, each cultivar received 100 maize plants. Grains were planted in holes (20 per ridge, three grains per hole) and then reduced to one plant per hole. The Agricultural Extension Recommendations were followed for irrigation, fertilizer levels, and agronomic methods. During ploughing, superphosphate fertilizer (15% P_2O_5) was applied at a rate of 450 kg/ ha, and potassium sulphate (48% K_2SO_4) was applied at a rate of 125 kg/ha. Following planting, 300 N unit/ha of ammonium nitrate fertilizer (33.5% N) was applied in two treatments: the first before the first irrigation and the second before the second irrigation. Three weeks after planting, the first irrigation occurred. Irrigation was then scheduled every 12 days until it was completed three weeks before harvest. Weeds were removed by hand as soon as they appeared. The disease incidence of late wilt was monitored on a regular basis beginning 60 days after sowing, based on wilt symptoms, which included the following: infected plants' leaves turn pale green and

roll inward, as if suffering from a lack of water, and eventually become dry. The symptoms of drying progress upward, discoloring the stems to a yellow-brown hue. At the end of the experiment (110 days after planting), the percentage of infected plants with late wilt infection was calculated as follows: Late wilt infection (%) = [number of dead plants due to late wilt infection during the growing season/total number of maize plants] × 100. During the harvest period, quantitative maize yield was calculated by weighing the harvested ears per plot (kg).

Statistical analysis

The data were checked for normality and variance homogeneity before statistical analysis. Although percentage data was transformed using an arcsine square root transformation to improve variance homogeneity, untransformed data was presented. An analysis of variance (ANOVA) was run on all of the data to see if there was a significant difference in the mean of the estimated variables due to the use of beta-sitosterol and maize cultivars. Duncan's multiple range test was used in CoStat6303Win.exe programming to investigate the means at $P \leq 0.05$.

Results

In vitro antifungal activity of beta-sitosterol

The antifungal effect of beta-sitosterol on *C. maydis* colony diameter was evaluated in vitro using the culture plate sensitivity assay at various concentrations of 50, 100, 150, 200, and 250 ppm (Figs. 2 and 3). When compared to untreated plates, beta-sitosterol significantly reduced the colony diameter of *C. maydis* on culture plates (Fig. 3). The inhibitory effect of beta-sitosterol in agar plates increased as the concentration of beta-sitosterol in the plates increased (Fig. 3). *C. maydis* growth was completely inhibited by beta-sitosterol at 250 ppm. A spore germination assay was used to investigate the effect of beta-sitosterol on *C. maydis* early development (Fig. 3). In beta-sitosterol-containing growth conditions, pathogen spore germination was significantly reduced (Fig. 3). In fact, at the end of the incubation period, a high concentration of beta-sitosterol (250 ppm) inhibited the germination of 77.3% of fungal spores.

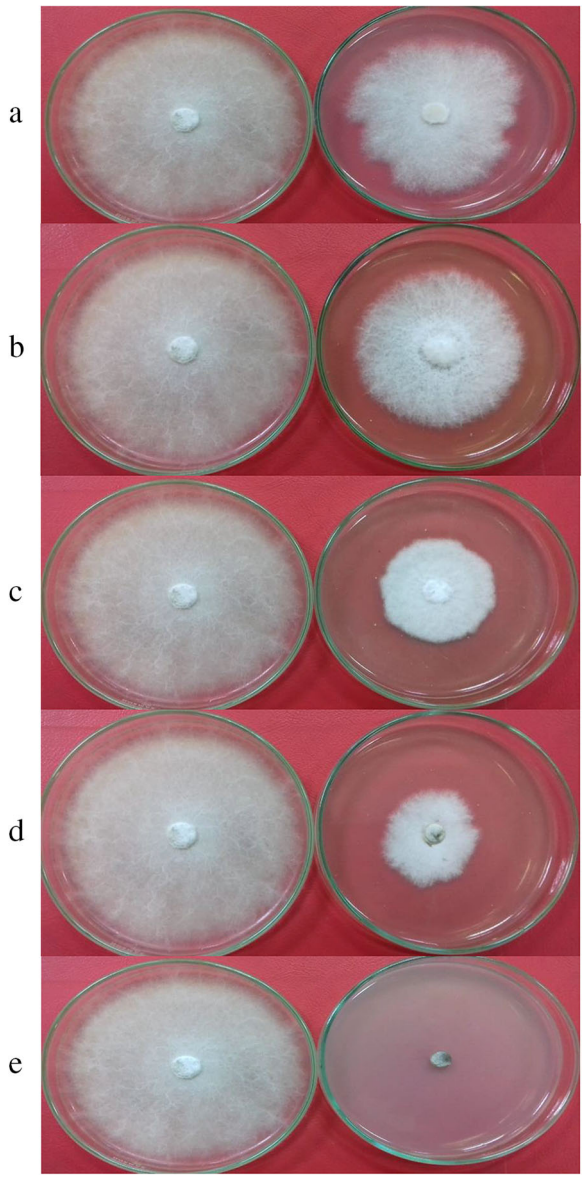

Fig. 2 The in vitro evaluation of beta-sitosterol efficiency against the colony of *Cephalosporum maydis* using agar plate assay. The beta-sitosterol was evaluated at a rate of 50 ppm (**a**), 100 ppm (**b**), 150 ppm (**c**), 200 ppm (**d**) and 250 ppm (**e**). Right plates- potato dextrose agar plates with beta-sitosterol. Left plates -potato dextrose agar plates without beta-sitosterol. Inhibition of *C. maydis* mycelial growth by beta-sitosterol was evaluated by measuring the colony diameter seven days after incubation at 28 ± 2 °C in the dark. Photographs were taken on the same day

Greenhouse experiments

The effect of beta-sitosterol treatments, maize cultivars, and their interaction on maize growth promotion (plant height cm plant^{-1}, fresh weight g plant^{-1}, and dry

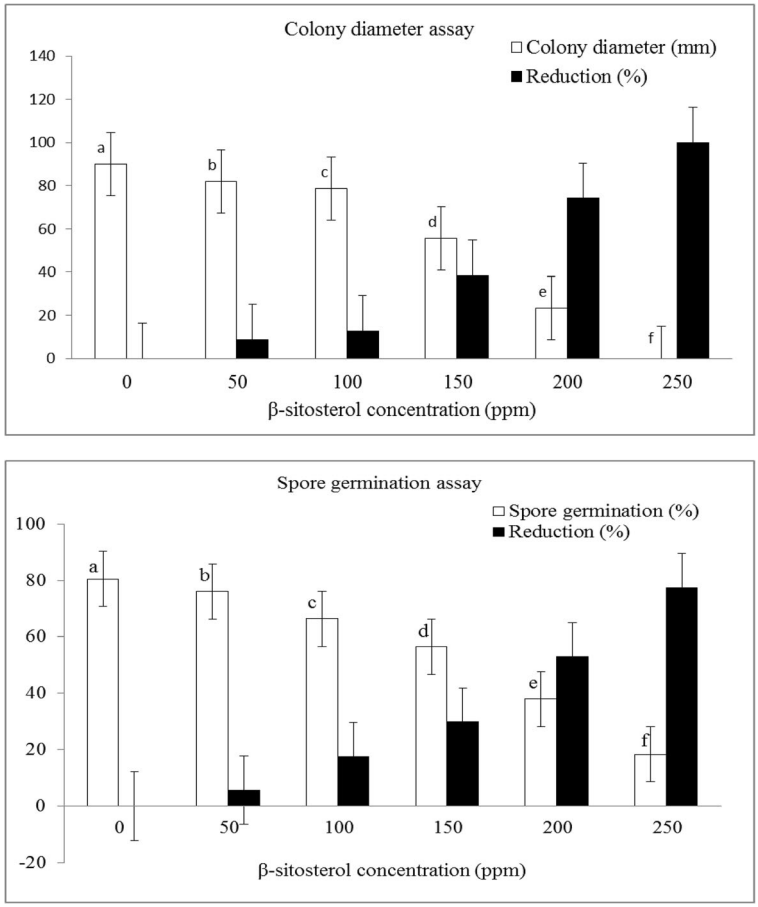

Fig. 3 Inhibition effects of different concentrations i.e., 0, 50, 100, 150, 200 and 250 ppm of beta-sitosterol against *Cephalosporum maydis* via colony diameter and spore germination assays under in vitro conditions. For colony diameter assay, agar disc (5 mm) from *C. maydis* was grown at 28 ± 2 °C on PDYA medium amended with beta-sitosterol. Fresh PDYA medium free from beta-sitosterol was used as control. Colony diameter (mm) was calculated 7 days after inculcation. For spore germination assay, conidial spores of *C. maydis* were grown at 28 ± 2 °C on PDYB medium amended with beta-sitosterol. Fresh PDYB medium free from beta-sitosterol was used as control. Spore germination (%) was calculated 18 h after inculcation. Values are mean of six replications for each beta-sitosterol concentration as well as the control. Bars with the same letters within each variable indicate that the means ± standard errors are not significantly different at $P \leq 0.05$, according to Duncan's multiple range tests

weight g plant^{-1}), induction defense enzymes (peroxidase, polyphenoloxidase, and chitinase), physiological parameters (chlorophyll contents, total phenols, and flavonoids), and late wilt incidence was summarized (Table 2) using an analysis of variance (ANOVA).

Effect of beta-sitosterol application on plant growth promotion

When compared to untreated controls, beta-sitosterol (250 ppm) had a significant impact on the growth parameters of maize plants from both cultivars (Fig. 4). Statistical studies, with the exception of dry weight, revealed no significant differences ($P \leq 0.05$) between cultivars and their interactions with treatments (Table 2). Beta-sitosterol application increased the dry weight of the Fine seed 1005 cultivar more than the Land race cultivar. The most effective treatment for Land race and Fine seed 1005 cultivars was beta-sitosterol grain soaking plus foliar spraying, which increased the average plant height, fresh weight, and dry weight of maize by 37.9 and 34.6, 32.2 and 35.1, and 72.2 and 73.7%, respectively. This was followed by beta-sitosterol grain soaking and/or foliar spraying, which increased average plant height, fresh weight, and dry weight by 24.6;26.9 and 21.6;21.8%, 17.4;17.9 and 23.1;23.1%, and 56.2;57.5 and 64.5;64.9%, respectively.

Effect of beta-sitosterol application on induction defense enzymes

In contrast to the control, the application of beta-sitosterol (250 ppm) increased the activity of defense enzymes (peroxidase, polyphenoloxidase, and chitinase) in treated maize plants (Fig. 5). There is a statistically significant ($P \leq 0.05$) difference between cultivars and their interactions with beta-sitosterol treatments (Table 2). When beta-sitosterol was applied, the Fine seed 1005 cultivar's mean enzymatic activities

Table 2 Summary of the analysis of variance for the effect of beta-sitosterol application on maze growth promotion traits, induction defense enzymes traits and late wilt incidence studies under greenhouse conditions

Source of variation	df	Mean Square		
		Plant height	Fresh weight	Dray weight
Main plots				
Blocks	5	46.11^{ns}	101.73667^{ns}	3.3066667^{ns}
Cultivars (C)	1	50.416667^{ns}	50.416667^{ns}	355.26667^{***}
Main Plot Error	5	22.856667	25.176667	8.7066667
Subplot				
Treatments (T)	4	2638.4333^{***}	2233.7083^{***}	2955.7333^{***}
C×T	4	20.833333^{ns}	20.208333^{ns}	42.516667^{***}
Error	40	35.333333	30.198333	11.215
Source of variation	df	Mean Square		
		Peroxidase	Polyphenoloxidase	Chitinase
Main plots				
Blocks	5	2.764^{ns}	3.1886^{ns}	1.0363^{ns}
Cultivars (C)	1	0.0423473^{***}	0.0523921^{***}	0.1514033^{***}
Main Plot Error	5	2.3707	1.1851	7.0247
Subplot				
Treatments (T)	4	0.0580282^{***}	0.1345863^{***}	0.9089615^{***}
C×T	4	0.0092779^{***}	0.0062471^{***}	0.0224542^{***}
Error	40	2.1948	2.1801	6.8251
Source of variation	df	Mean Square		
		Chlorophyll	Phenols	Flavonoids
Main plots				
Blocks	5	0.0190667^{ns}	0.0352^{ns}	1.4567^{ns}
Cultivars (C)	1	0.5606667^{***}	6.8006667^{***}	3.9015^{***}
Main Plot Error	5	0.0438667	0.0642667	2.71
Subplot				
Treatments (T)	4	3.061^{***}	171.04067^{***}	0.0080817^{***}
C×T	4	0.1356667^{***}	0.8365^{***}	8.0667^{ns}
Error	40	0.0221333	0.1009833	4.325
Source of variation	df	Mean Square		
		Late wilt incidence		
Main plots				
Blocks	5	1841.6667^{ns}		
Cultivars (C)	1	375^{ns}		
Main Plot Error	5	375		
Subplot				
Treatments (T)	4	11000^{***}		
C×T	4	166.66667^{ns}		
Error	40	358.33333		

were significantly ($P \leq 0.05$) higher than those of the Land race cultivar. The most effective treatment for each cultivar was beta-sitosterol grain soaking plus foliar spraying. Treatments involving beta-sitosterol grain

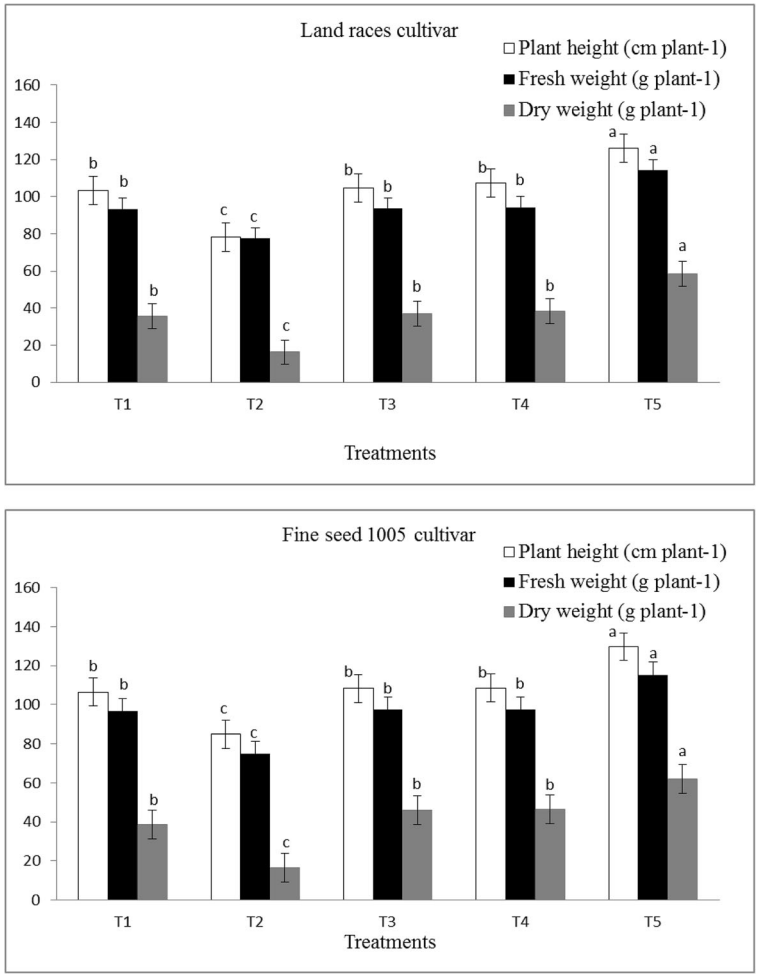

Fig. 4 Effect of application with beta-sitosterol (250 ppm) on plant growth promotion of maize plants, 35 days after sowing, grown under greenhouse conditions. Negative control (T1), positive control (T2), beta-sitosterol soaking (T3), beta-sitosterol spraying (T4), beta-sitosterol soaking plus spraying (T5). Values are mean of six replications for each treatment as well as the positive and negative controls. Bars with the same letters within each variable indicate that the means± standard errors are not significantly different at $P \leq 0.05$, according to Duncan's multiple range tests

soaking and/or beta-sitosterol foliar spraying were closely followed. When beta-sitosterol grain soaking was combined with foliar spraying, the activities of peroxidase (39.4 and 21.3 times), polyphenoloxidase (30.5 and 27.2 times), and chitinase (41.4 and 42.6 times) were significantly higher in the Land race and Fine seed 1005 cultivars than the control (Fig. 5).

Effect of beta-sitosterol application on physiological parameters

When compared to the control, the application of beta-sitosterol (250 ppm) resulted in a significant increase in physiological parameters (chlorophyll content, total phenols, and flavonoids) in the treated maize plants (Table 3). There is a statistically significant ($P \leq 0.05$) difference between cultivars and their interactions with beta-sitosterol treatments (Table 2). When compared to the control, the mean values of physiological parameters of the Fine seed 1005 cultivar were significantly ($P \leq 0.05$) higher than those of the Land races cultivar. The most effective treatment for each cultivar, according to the results, was beta-sitosterol grain soaking plus foliar spraying. Treatments involving beta-sitosterol grain soaking and/or beta-sitosterol foliar spraying were closely followed. When compared to the control, beta-sitosterol grain soaking plus foliar spraying significantly increased total phenols (40.5 and 42.6 times), flavonoids (49.1 and 47.8 times), and total chlorophyll (31.3 and 30.6 times) in Land races and Fine seed 1005 cultivars, respectively (Table 3).

Effect of beta-sitosterol application on late wilt incidence

When compared to untreated controls, beta-sitosterol (250 ppm) application reduced the incidence of late wilt

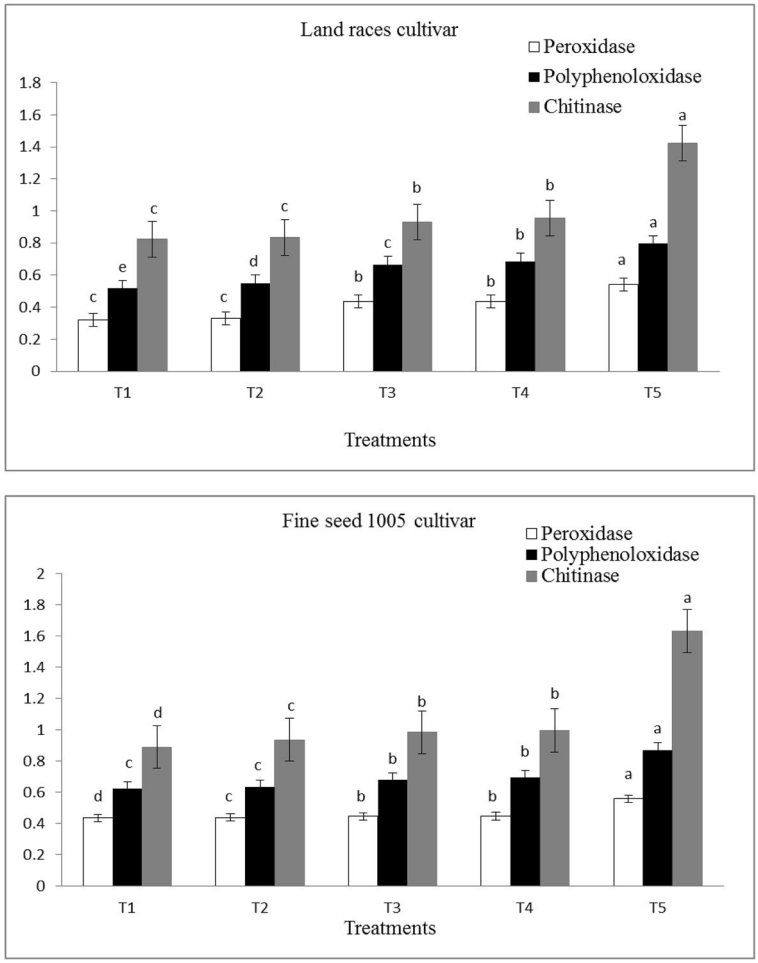

Fig. 5 Peroxidase, polyphenoloxidase and chitinase activities in maize leaves resulted from the application of beta-sitosterol (250 ppm), 35 days after sowing, grown under greenhouse conditions. Negative control (T1), positive control (T2), beta-sitosterol soaking (T3), beta-sitosterol spraying (T4), beta-sitosterol soaking plus spraying (T5). Values are mean of six replications for each treatment as well as the positive and negative controls. Bars with the same letters within each variable indicate that the means ± standard errors are not significantly different at $P \leq 0.05$, according to Duncan's multiple range tests. Peroxidase activity was expressed as the increase in absorbance at 470 nm/g fresh weight/ min. Polyphenoloxidase activity was expressed as the increase in absorbance at 475 nm/g fresh weight / minute. Chitinase activity was expressed as mM Nacetyl glucose amine equivalent released / gram fresh weight / 60 min at 540 nm

in maize plants of both cultivars (Fig. 6). According to statistical studies, there was no significant difference ($P \leq 0.05$) between cultivars and their interactions with beta-sitosterol treatments (Table 2). The beta-sitosterol grain soaking plus foliar spraying treatment reduced the incidence of late wilt by 25% in Land races and Fine seed 1005 cultivars, compared to 91.7 and 75.0% in the positive control treatment, respectively (Fig. 6). Treatments involving beta-sitosterol grain soaking and/or beta-sitosterol foliar spraying were closely followed.

Field experiments

The impact of beta-sitosterol treatments, maize cultivars, and their interactions on maize late wilt incidence and ear yield was described using an analysis of variance (ANOVA) (Table 4).

Effect of beta-sitosterol application on late wilt incidence

When compared to untreated controls, beta-sitosterol (250 ppm) application reduced the incidence of late wilt in maize plants of both cultivars (Fig.7). The statistical analysis revealed that maize cultivars, beta-sitosterol treatment techniques, and their interaction ($P \leq 0.05$) all had a significant impact on the incidence of late wilt in the field (Table 4). When beta-sitosterol was applied, the Fine seed 1005 cultivar's mean late wilt incidence was significantly ($P \leq 0.05$) lower than that of the Land race cultivar. Overall, the most effective treatment in this regard was a combination of beta-sitosterol grain soaking and foliar spraying (Figs. 8 and 9). It was then followed by beta-sitosterol grain soaking and/or foliar spraying. The treatment, which included grain soaking and foliar

Table 3 Phenols, flavonoids and total chlorophyll in maize leaves resulted from the application of beta-sitosterol, 35 days after maize planting under greenhouse conditions

Treatment	Chlorophyll (mg g^{-1} FW)	Phenols (mg g^{-1} DW)	Flavonoids (mg g^{-1} DW)
Land races cultivar			
T1	3.23±0.05 c	10.5±0.08 e	0.047±5.16 e
T2	2.63±0.06 d	11.6±0.13 d	0.057±4.77 d
T3	3.45±0.13 b	13.4±0.10 c	0.078±5.57 c
T4	3.75±0.04 a	15.4±0.10 b	0.088±3.33 b
T5	3.83±0.02 a	19.5±0.16 a	0.112±3.65 a
Fine seed 1005 cultivar			
T1	3.10±0.06 c	10.9±0.03 e	0.053±8.72 e
T2	2.88±0.01 d	12.1±0.18 d	0.061±0.00 d
T3	3.87±0.02 b	13.8±0.05 c	0.081±0.00 c
T4	3.87±0.02 b	15.9±0.04 b	0.096±8.81 b
T5	4.15±0.08 a	21.1±0.19 a	0.117±6.14 a

Negative control (T1), positive control (T2), beta-sitosterol soaking (T3), beta-sitosterol spraying (T4), beta-sitosterol soaking plus spraying (T5). Values are mean of six replications for each treatment as well as the positive and negative controls. Means ± standard errors within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

spraying, reduced late wilt by 83.1% in the Land race cultivar and 76.6% in the other cultivar.

Effect of beta-sitosterol application on the ear yield

When compared to untreated controls, beta-sitosterol (250 ppm) had a significant impact on the ear yield of maize plants of both cultivars (Fig. 10). Statistical analysis revealed that maize cultivars, beta-sitosterol treatment techniques, and their interaction ($P \leq 0.05$) all had a significant effect on maize ear yield in the field (Table 4). Under the same conditions, maize plants of the Fine seed 1005 cultivar treated with beta-sitosterol in various methods had significantly higher ear yields ($P \leq 0.05$) than those of the Land races cultivar. The best results were obtained by combining beta-sitosterol grain soaking and foliar spraying, which increased the average weight of ears per plot by 61.0% in the Land race cultivar and by 56.3% in the Fine seed 1005 cultivar (Fig. 9). Individual beta-sitosterol grain soaking or foliar spraying came in second, increasing average ears weight per plot by 33.1 and 36.4% in Fine seed 1005 cultivar, respectively, and by 26.5% in Land race cultivar.

Discussion

The soil-borne and seed-borne fungus *C. maydis* causes late wilt disease, which has a significant impact on maize production worldwide, resulting in lower yields and quality. At this moment, synthetic fungicides with a good antifungal effect against *C. maydis* are the best option for controlling this disease (Degani et al., 2018). However, new disease prevention strategies that adhere to sustainable agriculture standards are required. The phytosterol-related bioorganic molecule beta-sitosterol has a long history of use as a food and medication and is generally thought to be safe and free of side effects (Saeidnia et al., 2014). The structure of beta-sitosterol is similar to that of cholesterol and ergosterol, two important components of mammalian and yeast cell membranes (Saeidnia et al., 2014). Several researchers have recently used beta-sitosterol to reduce the negative effects of various abiotic stressors (Divi & Krishna, 2009; Elkeilsh et al., 2019; Yenjit et al., 2010). As a result, we used this nontoxic chemical as an antifungal agent against *C. m*aydis in vitro and as a resistance inducer against maize late wilt in vivo. *C. maydis* mycelium and spores are present in the host plant xylem vessels during pathogenesis and may be exposed to the host environment (Samra et al., 1963). As a result, the mycelium and/or spore germination test may be a useful tool for determining the effect of various environmental and host conditions on pathogen development and spread (Degani et al., 2014).

Beta-sitosterol demonstrated promising antifungal activity in vitro against *C. maydis* mycelial development and spores in this study. All of the concentrations tested were inhibitory, but the inhibition of *C. maydis* colony diameter and spore germination increased with increasing beta-sitosterol concentration. The higher density of beta-sitosterol particles may help with this because they can absorb and adhere to pathogen fragments, effectively deactivating the growth. This study's findings are consistent with those discovered by Aderiye et al. (1989). They discovered that the antifungal activity of beta-sitosterol compound suppressed fungal spore germination by approximately 40% at a concentration of 50 μg/ml. According to Ueda et al. (1990), phytosterols such as stigmasterol, campesterol, and sitosterol have in vitro antifungal activity against *Fusarium oxysporum* f. sp. *cucumerinum*. Furthermore, Aderiye et al. (1996) found that beta-sitosterol had promising antifungal activity against *Fusarium moniliforme*. *Salmonella typhii*, *Corynebacterium*

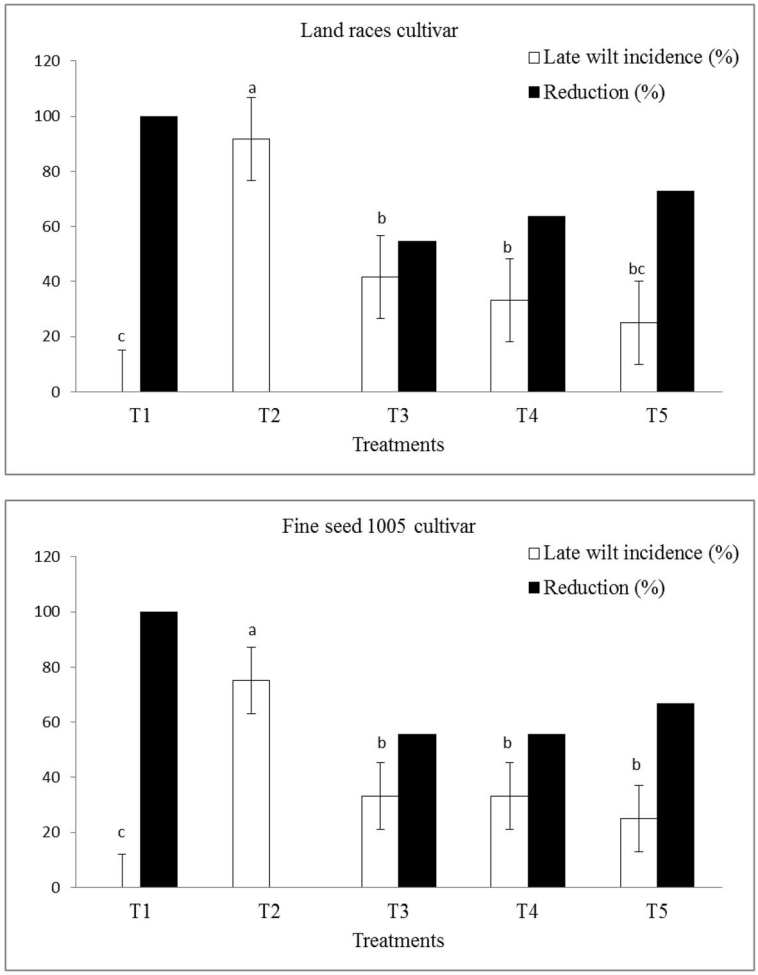

Fig. 6 Effect of beta-sitosterol (250 ppm) application on the incidence of late wilt of maize plants, 90 days after sowing, grown under greenhouse conditions. Negative control (T1), positive control (T2), beta-sitosterol soaking (T3), beta-sitosterol spraying (T4), beta-sitosterol soaking plus spraying (T5). Values are mean of six replications for each treatment as well as the positive and negative controls. Bars with the same letters within each variable indicate that the means± standard errors are not significantly different at $P \leq 0.05$, according to Duncan's multiple range tests. Percentages data of late wilt disease incidence (dead plants due to late wilt infection) were transformed into arcsine square root transformed data for analyses of variance; however, untransformed data are present

diphtheriae, *Vibrio cholerae*, *Fusarium* spp., and *Penicillium* spp. were all inhibited by beta-sitosterol, according to Kiprono et al. (2000). Beta-sitosterol extracted from the root bark of *Eucleanata lensis* effectively inhibited *Aspergilus niger* and *Cladosporium cladosporioides* (Lall et al., 2006). Yenjit et al. (2010) discovered that a mixture of stigmasterol and beta-sitosterol isolated from the pericarp of *Areca catechu* inhibited spore germination (ED_{50} 86.9 μg/mL), mycelial development (ED_{50} 56.7 μg/mL), and germ-tube elongation (ED_{50} 50.0 μg/mL) in *Colletotrichum gloeosporioides*. Furthermore, they claimed that this combination was more effective in vivo than the fungicide benomyl at controlling anthracnose disease in mango fruit. In vitro, Choi et al. (2017) discovered that crude extracts of *Dipsacus asper* roots and bioactive compounds such as beta-sitosterol inhibited the growth of *Botrytis cinerea*, *Colletotrichum coccodes*, *Blumeria graminis* f. sp. *hordei*, *Magnaporthe grisea*, *Phytophthora infestans*, *Puccinia recondita* and *Rhizoctonia solani*.

Table 4 Summary of the analysis of variance for the effect of beta-sitosterol application on maize late wilt incidence and ear yield studies under field conditions

Source of variation	df	Mean Square	
		Late wilt incidence	Yield
Main plots			
Blocks	3	1.9981909^{ns}	0.722567^{ns}
Cultivars (C)	1	541.82424^{***}	193.05406^{***}
Main Plot Error	3	7.2881069	0.0726832
Subplot			
Treatments (T)	3	1247.1067^{***}	1013.6222^{***}
C×T	3	106.21784^{***}	4.0764788^{***}
Error	18	2.2662037	0.5852547

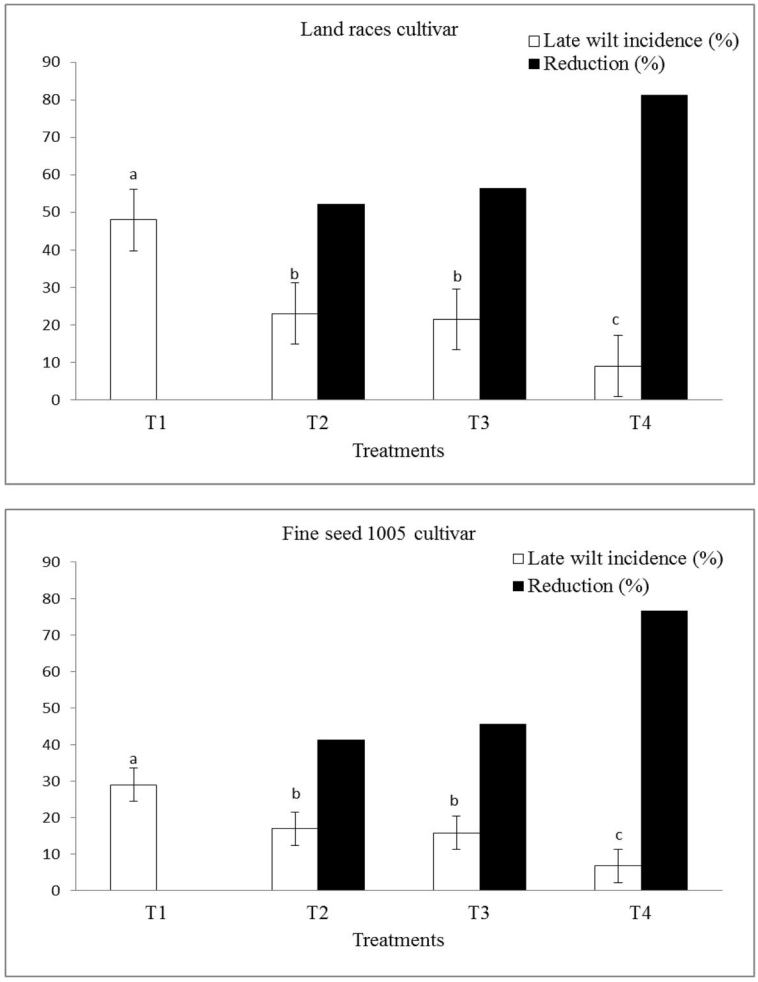

Fig. 7 Effect of beta-sitosterol (250 ppm) application on the incidence of late wilt of maize plants, 110 days after sowing, grown under field conditions. Untreated control (T1), beta-sitosterol soaking (T2), beta-sitosterol spraying (T3), beta-sitosterol soaking plus spraying (T4). Values are mean of four replications for each treatment as well as the control. Bars with the same letters indicate that the means± standard errors are not significantly different at $P \leq 0.05$, according to Duncan's multiple range tests. Percentages data of late wilt disease incidence (dead plants due to late wilt infection) were transformed into arcsine square root transformed data for analyses of variance; however, untransformed data are present

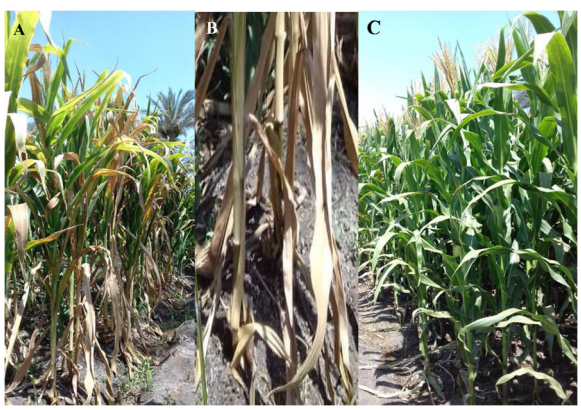

Fig. 8 Late wilt disease symptoms on Land races cv. plants under field trails 90 days after sowing. **A**, **B** un-treated control and **C** beta-sitosterol (250 ppm) grain soaking plus foliar spraying. Late wilt diseased field symptoms: drying out ascends upwards in the plant, including leaf yellowing and dehydration, and color alteration of the lower stem and internode

The precise mechanism of beta-sitosterol action in fungal pathogens is unknown. Botanical pesticides cause damage to fungal cell walls, membranes, and organelles (Yoon et al., 2013). These metabolites also inhibit spore germination, mycelial development, germ tube elongation, and delayed sporulation, as well as the synthesis of critical enzymes, DNA, and proteins (Yoon et al., 2013). Plant chemicals also cause underlying changes in the hypha and mycelia, which prevent pathogenic fungi such as *Aspergillus* spp. and *Fusarium* spp. from producing pathogenic substances like aflatoxin and fumonisin (Yoon et al., 2013). According to a new study, beta-sitosterol could be used to target yeast oxysterol-binding proteins (OSBPs) (Campos et al., 2014). These proteins are involved in the distribution and control of sterols, as well as cell signaling (Lehto & Olkkonen, 2003). Sterol is required for membrane transport as well as cell signaling (Dufourc, 2008).

Fig. 9 Late wilt disease symptoms on Fine seed 1005 cv. plants under field trails 90 days after sowing. **A**, **B** un-treated control and **C** beta-sitosterol (250 ppm) grain soaking plus foliar spraying. Late wilt diseased field symptoms: drying out ascends upwards in the plant, including leaf yellowing and dehydration, and color alteration of the lower stem and internode

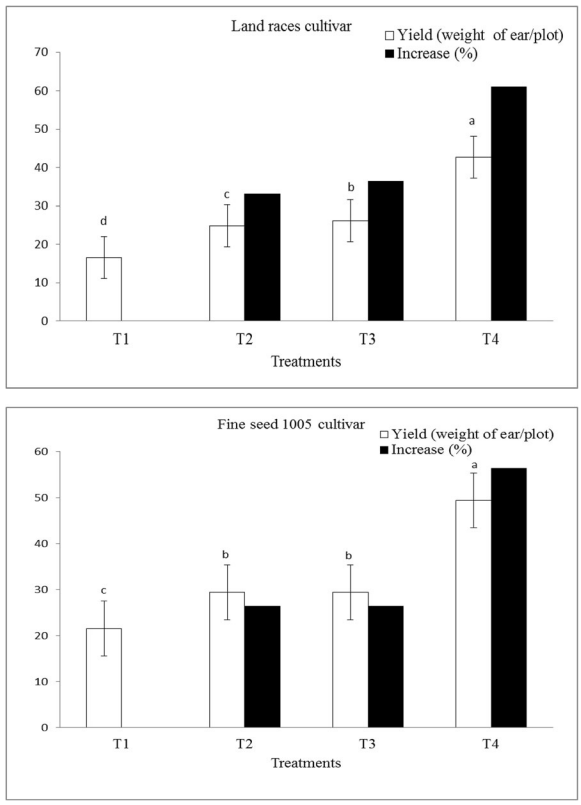

Fig. 10 Effect of beta-sitosterol (250 ppm) application on maize ear yield (kg ear/plot) of maize plants, 110 days after sowing, grown under field conditions. Untreated control (T1), beta-sitosterol soaking (T2), beta-sitosterol spraying (T3), beta-sitosterol soaking plus spraying (T4). Values are mean of four replications for each treatment as well as the control. Bars with the same letters indicate that the means ± standard errors are not significantly different at $P \leq 0.05$, according to Duncan's multiple range tests

Surprisingly, a commercial oomycetes fungicide, Oxathiapiprolin, targets OSBPs (Pasteris et al., 2016). OSBPs are Osh proteins that are encoded by the yeast *Saccharomyces cerevisiae's* seven Osh genes. Although Osh1, the major Osh protein, is incapable of maintaining normal cell viability on its own, the presence of at least one Osh protein is required (Beh et al., 2001). Beta-sitosterol concentrations, as well as Osh4 (an oxysterol-binding protein) and its homologs within fungal cells, are the primary determinants of beta-sitosterol poisonousness, according to Moosavi et al. (2020). They also stated that sterol uptake by fungal cells could contribute to beta- sitosterol's antifungal effect.

In this study, beta-sitosterol at 250 ppm reduced the incidence of maize late wilt disease in both greenhouse and field trails. The Fine seed 1005 cultivar, on the other hand, showed significantly fewer signs of late wilt disease than the Landraces cultivar. In both cultivars, the dual beta-sitosterol solution treatment, which included maize grain dipping and foliar spraying, was the most effective in reducing disease incidence. This decrease could be attributed to beta- sitosterol's direct antifungal activity, as well as indirect effects such as the induction of defense enzymes and the amount of total phenols and flavonoids present as a result of beta-sitosterol application. In maize leaves 35 days after planting, beta-sitosterol treatments significantly increase the specific activity of defense-related enzymes such as peroxidase, polyphenoloxidase, and chitinase. Increased peroxidase and polyphenoloxidase activity may lead to increased oxidative stress due to increased H_2O_2 production (Vance et al., 1980). H_2O_2 and other free radicals are poisonous to a wide range of microbial pathogens (Wu et al., 1995). During plant-pathogen interactions, the oxidative potential of H_2O_2 contributes to the formation of lignin via peroxidase-mediated cross-linking of proline-rich structural proteins and phytoalexin biosynthesis, as well as the conversion of O-dihydroxyphenols to toxic o-quinones via polyphenoloxidase (Mayer & Harel, 1997). Chitinase enzymes, on the other hand, aid in plant defense against pathogenic fungi by hydrolyzing cell walls. Because chitin is a major structural component of many pathogenic fungi's cell walls, their amounts increase significantly, and they play an important role in fungal

pathogen defense by degrading cell walls. Similar to our findings with beta-sitosterol, the highest concentration of brassinosteroids improved the antioxidant enzymes of cowpea plants (Lima & Lobato, 2017). Elkeilsh et al. (2019) discovered that application of beta-sitosterol to water-stressed wheat plants significantly increased the activity of antioxidant enzymes. According to Shahzad et al. (2021), the enhanced oxidative stress tolerance of beta-sitosterol-treated rice plants under stress was attributed to low levels of reactive oxygen species (ROS) and a significant increase in antioxidant enzyme activities (superoxide dismutase SOD, peroxidase POD, catalase CAT, and ascorbate peroxidase APX). Flavonoids play an important role in preventing diseases from infecting plants. They inhibit the production of ROS by pathogens and plants during infection (Dai et al., 1996). Furthermore, flavonoids can aid in plant structure and tissue fixation by influencing auxin action, which can promote tissue differentiation, callus and tylose growth, and the vascular system, all of which can help to prevent pathogen infection (Beckman, 2000). They may also play a direct role in inhibiting pathogen enzymes, particularly those that process plant cell walls, by chelating metals required for their function (Treutter, 2005). The phenolic and flavonoid contents of both maize cultivars were generally comparable to those of corresponding negative control treatments in various treatments of combined beta-sitosterol application and *C. maydis* inoculation in the current study. Phenolics are secondary metabolites that help plants defend themselves against a variety of pathogens (Taheri & Tarighi, 2011). Furthermore, phenolics act as radical scavengers, lowering the toxicity of reactive oxygen species (ROS) (Shoaib et al., 2018). The phenolic and flavonoid content of the maize leaves increased in the positive control treatment, possibly in response to pathogenic pressure that disrupted nitrogen availability. Doley and Jite (2013) discovered an increase in total phenolics in *Modiolula phaseolina*-infected groundnut plants. The accumulation of phenolic compounds at the challenged site may contribute to the localized accumulation of reactive oxygen species (ROS), triggering a general plant defense response (Doley & Jite, 2013). As a result, increasing enzyme activity was insufficient to deal with the increased ROS concentration, whereas ROS may facilitate pathogen penetration by increasing the activities of cell wall degrading enzymes, weakening the host defense system (Doley & Jite, 2013).

Structure resistance and assisting plants with scabbing from infection are two possible mechanisms by which beta-sitosterol administration protects maize plants from late wilt infection. Because late wilt disease appears at a late phonological stage of plant development (shortly before ripening), when auxin emission decreases and ethylene discharge increases (Shehata, 1976), foliar beta-sitosterol application may be involved in controlling late wilt development, which explains why it is effective. On the one hand, beta-sitosterol has been linked to plant part repair. According to Clouse and Sasse (1998), brassinosteroids, as steroidal chemicals, accelerated cell wall construction, hyperpolarized cell membranes, and accelerated the growth cycle. Sitosterol and spermidine stimulated vascular bundle development and increased stem diameter/cross section, ground tissue thickness, and pith cavity width in wheat plants, according to Abd El-Wahed et al. (2001). Rice plants sprayed twice with 150 ppm stigmasterol increased leaf thickness, upper and lower epidermal layers, mesophyll tissue, and measurements of both main and smaller leaf vascular bundles, according to Ali et al. (2002). Nassar (2004) discovered that stigmasterol increased the thickness of the soybean plant's epidermis, cortex, vascular cylinder, palisade, and spongy tissues. According to Helal and Gomaa (2007), treating Egyptian lupine plants with 80 ppm stigmasterol caused significant changes in the anatomical structure of the main stem due to increased thickness of most involved tissues. When compared to the control, Nassar et al. (2013) discovered that foliar treatment with 90 ppm stigmasterol increased the diameter of flax main stems at their median section by 17.3%. The epidermis, cortex, fibrous area, secondary phloem and xylem tissue, and pith diameter were all 5.6, 47.1, 20.2, 14.1, 30.0, and 8.1% thicker, respectively, than the control. Wheat plants treated with beta-sitosterol (10^{-5} M) showed ultrastructure changes, including a significant increase in cell volume, a thicker cell wall, and a larger vacuole than untreated plants, according to Gamel et al. (2017). El-Tantawy and Azoz (2019) discovered that applying 100 ppm stigmasterol to basil plants increased the breadth of the main stem by 30.20% when compared to the control. They also stated that the increase in basil plant stem diameter caused by 100 ppm stigmasterol foliar application was due to a noticeable increase in thickness of all included tissues, including pith diameter, cortex thickness, fiber strands, phloem tissue, and xylem tissue, as well as pith diameter, which increased

by 33.3, 25.8, 20.7, 56.9, and 5.9% more than those in untreated plants' stems. Furthermore, when compared to the control, the vessel diameter increased by 26.4%.

Finally, under greenhouse and field conditions, beta-sitosterol at 250 ppm increased maize plant vegetative growth and ear yield, respectively. The most effective treatment was a combination of maize grain dipping and foliar spraying. This effect could be attributed to beta-sitosterol's role in the development of vegetative growth properties. Beta-sitosterol boosts water uptake and utilization efficiency, as well as cell division and expansion, resulting in longer shoots and more leaf area, which increased shoot dry matter, most likely due to more surface area available for anabolic exercises (Hashem et al., 2011). One possible explanation for the increase in yield production in plants treated with exogenous beta-sitosterol spraying is better use of light energy and increased photosynthesis, which results in more dry matter and, consequently, higher yields (Jiang et al., 2013; Kaya et al., 2018; Vriet et al., 2012). Under greenhouse conditions, the use of beta-sitosterol at 250 ppm increased the amount of total chlorophyll in maize leaves in this study. Pathogen inoculation, on the other hand, decreased chlorophyll content in the current study, possibly due to xylem vessel choking caused by fungal toxin release (García-Carneros et al., 2011). The improved photosynthetic pigmentation of the leaf as a result of beta-sitosterol application could be attributed to a decrease in fungal toxin release by pathogens. Sitosterol stimulated vegetative growth parameters (shoot length, leaf area, plant fresh and dry weights) in wheat, according to Abd El-Wahed et al. (2001). Abd El-Wahed (2001) discovered that sitosterol treatment caused maize plants to form roots. Adding sitosterol to cotton cuttings stimulates root production and improves cotton plant growth and productivity, according to Abd El-Wahed and Mekki (2011). Soliman et al. (2016) discovered that 100 ppm cytokinins and 10^{-5} M beta-sitosterol significantly increased mung bean plant growth metrics such as root length, shoot length, root fresh and dry weight, shoot fresh and dry weight, and number of leaves per plant. According to Gamel et al. (2017), beta-sitosterol treatment increased the number and area of leaves in tomato plants. Elkeilsh et al. (2019) discovered that using 100 mg L^{-1} beta-sitosterol improved the growth of water-stressed wheat crops. According to El-Tantawy and Azoz (2019), applying stigmasterol improved basil plant growth and productivity, as well as the proportion and composition of volatile oil, with 100 ppm stigmasterol showing the greatest improvement. According to Shahzad et al. (2021), the application of beta-sitosterol to rice plants improves numerous physiological parameters associated with growth and development, such as shoot and root length, whole plant biomass, chlorophyll pigments, and photosynthetic-related parameters, when compared to non-treated plants.

Conclusion

The findings show that beta-sitosterol, a resistance inducer, inhibits the fungal colony formation and/or spore germination of the maize late wilt pathogen *C. maydis* in vitro. The use of beta-sitosterol (250 ppm) significantly reduced the incidence of late wilt of maize in greenhouse and field conditions. There were three methods of application used, with grain soaking plus foliar spraying proving to be the most effective. The effects of beta-sitosterol on maize plant defense against late wilt infection are expected to increase total phenol, total flavonoids, antioxidant enzymes, and photosynthetic pigments, among other mechanisms. As a result, the safe resistance inducer beta-sitosterol could be used to boost maize plant resistance to *C. maydis* infection.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement All authors have approved the manuscript and agreed with its submission to European Journal of Plant Pathology. The submitted work is original and have not been submitted or published elsewhere. The manuscript has been prepared following principles of ethical and professional conduct. The study does not involve human participants or animals.

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