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Using maize wastes, fermented by co-cultures of *Trichoderma harzianum* and *Pseudomonas fluorescens*, as grain dressing against m maize diseases under field conditions

Sherien M. M. Atalla^{1*}, Mokhtar M. Abdel-Kader², Nadia G. El-Gamal² and Nehal S. El-Mougy²

Abstract

Maize (*Zea mays* L.) is one of the most economic crops in Egypt. Production of amylase from the waste of maize is the most economic and cheap renewable and most abundant raw materials present in environment. Biosynthesis of Cu-chitosan nanoparticles for amylase production by co-culturing between *Trichoderma harzianum* and *Pseudomonas fluorescens* at different ratios compared to free conditions was the main purpose of this study. The optimum ratio 8:2, recorded between *P. fluorescens*: *T. harzianum*, was the most promising for production of amylase produce 22.47 and 28.60 U/ml for free and nano, respectively. The UV visible spectral analysis Cu-chitosan NPs was 220 nm, while the mean diameter, using transmission electron microscopy was 0.5 μ m. Application of fermented maize wastes by co-cultivation of *P. fluorescence* and *T. harzianum*, as a grain dressing before sowing declared the reduction in both root and foliar diseases during the maize growing season, starting from germination up to 70 days of its vegetative growth under field conditions. A promising approach is the creation and use of environmentally safe products, whose protective effect is based on the induction of hydrolase inhibitors in plants.

Keywords: Maize wastes, Maize diseases, Amylase, Co-culturing, Cu-chitosan nanoparticles, *Trichoderma harzianum*, *Pseudomonas fluorescens*

Background

Maize (*Zea mays* L.) is one of the most important cereal crop in Egypt and worldwide. Maize attacked by many fungi causing serious diseases. Root rot, leaf spots, late wilt, and ear rots are major diseases reflected on plant stand and product yield. Since there are no resistant maize cultivars against pathogenic fungi as well as the fungicides are not effective enough to keep the crop safe from harm or injury by these pathogens (Lucas et al. 2015), therefore,

development of biocontrol agents could be one of the available possibilities for reducing the incidence of these diseases. Recently, scientists have great attention towards using biocontrol agents against plant diseases (Elad and Stewart 2007). Agricultural crops are attacked by plant pathogens. The activity of hydrolytic enzymes of these pathogens determined their capability to invade and disperse in plant tissues (Silva et al. 2013). The hydrolytic enzymes secreted by pathogens participate in starting and developing of plant diseases. Therefore, effective mechanism for preventing the invasion and spreading of pathogens in plant tissues is by using specific inhibitors to suppress the action of hydrolase activity (Misas-Villami and Horm 2008). These inhibitors may act directly to

* Correspondence: atallams@yahoo.com

¹Chemistry of Natural and Microbial Products Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki, P.O. Box: 12622, Giza, Egypt
Full list of author information is available at the end of the article

deactivate the alien enzymes of invading pathogen or indirectly by increase plant resistance defense mechanisms (Gatehouse, 2011).

Maize wastes consist of various chemicals, including proteins, vitamins, alkaloids, tannins, and mineral salts carbohydrates, steroids, flavonoids, and volatile chemicals (Velazquez et al. 2005; El-Ghorab et al. 2007).

Amylases are a group of hydrolase, reduce the viscosity of starch by breaking down 1-4- α -D-glucosidic bonds to produce varied sized chains of glucose. The families of amylase enzymes are of great significance due to their areas of potential applications as additives to detergents to remove stains in high fructose corn syrup preparations, brewing, fermented drink industry, scarification of starch for alcohol production, and in the production of adhesives; also amylase is used as possible application in several manufacturing procedures such as food, textiles, and paper industries (Pandey et al. 2003). Microbial amylases from fungi and bacteria have successfully replaced chemical hydrolysis of starch in starch processing industries. They would be potentially useful in fine chemical industries and pharmaceutical process (Vasant 2018). The potential of using microorganisms as sources of industrially relevant enzymes has stimulated interest in the exploration of extracellular enzyme activities in several microorganisms (Ominyi 2013). The other advantage of filamentous fungi in amylase production is that the fungal mycelia that easy cultivation, synthesize, and release large quantities of extra-cellular hydrolytic enzymes (Manpreet et al. 2005). Isolation of new microbial amylases from fungi is important for providing capability, new sources of enzyme (Ahmed et al. 2019).

Co-cultivation or mixed cultivation is the one that imitates the natural habitation where microbes co-exist within complex microbial communities; also co-cultures enable to combine metabolic activity in the laboratory conditions for better utilization of substrates either by antagonistic/ symbiotic interactions (Vigneshwari et al. 2017). The use of low cost substrates was the best for co-culture in enzyme production (Furukawa et al. 2013).

Nanotechnology plays a remarkable role in modern agriculture to address universal challenges, especially from fungi, which act as tolerant metal bioaccumulation, thus they are more attracted on biological production of metallic nanoparticles. Nanoparticles used in plant disease management (Sharma et al. 2016) increase the availability of important plant nutrients; higher crop production reduce the continued use of chemicals in agriculture increases environmental pollution, cost of food production, and unfavorable side effects (Parisi et al. 2015).

The use of chitosan is a promising polymers consisting of glucosamine and N-acetyl glucosamine units obtained from the waste of industrial fishing activities (Saharan and Pal 2016; Gomes and Paschoalin, 2017) Chitosan-

based nanoparticles (NPs) are known for nontoxic, safe, biodegradable, and biocompatible (Saharan et al. 2015). Copper as transition metal is usually utilized in organisms as a cofactor in enzymes or electron transfer proteins through redox reactions or oxygen chemistry. Cu is most important micronutrient which required for plant protection and growth. It also acts as active components in several enzyme protein synthesis, metabolism of carbohydrates, and gene expression (Badawy and Rabea 2011; Rajasekaran and Santra 2015; Choudhary et al. 2017). Many applications of chitosan nanoparticles have been extensively studied to overcome the major problems associated with enzyme production, conventional methods in agriculture also can be used as antifungal and antibacterial (Chen et al. 2014).

The aim of the present work was the synthesis of Cu-chitosan nanoparticles and its applications on amylase production by co-cultivation of *Trichoderma harzianum* and *Pseudomonas fluorescens* in comparison of free/nanoparticles grown on maize wastes. The synthesis of Cu-chitosan NPs grains dressing of fermented maize wastes by co-cultivation of the tested antagonistic microorganisms against maize diseases under field conditions was also evaluated.

Materials and methods

Microorganisms

The fungus *Trichoderma harzianum* and the bacterium isolate *Pseudomonas fluorescens* were kindly obtained from the Culture Collection Unit, Plant Pathology Department, National Research Centre, Giza, Egypt. These 2 isolates proved their highly antagonistic ability against various plant pathogens (El-Mougy et al. 2016). These isolates were kept at 4 °C on suitable media, i.e., potato dextrose agar and nutrient agar (Sigma Aldrich Spruce Street, St. Louis, USA) for the fungus and bacterium, respectively.

Sample collection

Maize fresh wastes were collected from different fields located at El-Saff district, Giza Governorate, Egypt, and cut into small pieces (cubes 1 cm in diameter) reaching a constant weight until constant weight is reached.

Inoculation and fermentation media conditions

Fermentation was carried out in 250 ml Erlenmeyer flasks each containing 50 ml of fermentation media consists of the following (g/l): maize waste (20.0), K_2HPO_4 (1.0), $MgSO_4$ (0.5) KCl (0.5), $FeSO_4$ (0.01), and autoclaved at 121 °C for 15 min (El-Shamy et al., 2016). One ml of 10^6 spore suspensions of *T. harzianum*/flask was inoculated and incubated at 28 ± 2 °C at 200 rpm. While one ml cell suspension 10^6 of *P. fluorescens*/flask was inoculated and incubated at 28 ± 2 °C at 200 rpm. In

addition to mixed cultures of both *T. harzianum* and *P. fluorescence* inoculated and incubated at $28 \pm 2^\circ\text{C}$ for 5 days. Five flasks were used for each microorganism.

Preparation and characterization of Cu-chitosan nanoparticles

Cu-chitosan NPs were synthesized from method based on ionic gelation of chitosan with sodium triphosphate (TPP) and CuSO_4 (Saharan et al. 2015). Briefly, 500 mg of chitosan was dissolved, using 1% (v/v) acetic acid and stirred at 200 rpm until solution becomes clear. Further, the TPP solution (5 mg/ml) was prepared and added to the chitosan solution gradually in drop wise manner under continuous stirring condition using a magnetic stirrer at room temperature ($20\text{--}22^\circ\text{C}$). Before completion of crosslinking reaction with TPP, CuSO_4 solution (2 mg/ml) was added. The resulting solution was centrifuged at 10,000 rpm for 15 min at 4°C , followed by sonication to achieve Cu-chitosan NPs. Nanoparticles were dried using freeze drying and stored for further use.

Amylase assay

Amylase assay was carried out using 4 ml of the reaction mixture (fungal and bacterial co-cultivation), which consisted of 1 ml of centrifuged enzyme solution and 2 ml of soluble starch in phosphate buffer, pH 6.5 (Wood and Bhat 1988). The mixture was incubated for 10 min at 30°C . Then the amount of reducing sugar was determined by dinitrosalicylic acid (Miller 1959) at 540 nm and expressed in units. One unit of enzyme is the amount of enzyme releasing 1 mg of glucose/ml/min.

Co-cultivations of *T. harzianum* and *P. fluorescence* on amylase production

To determine the influence of fungal and bacterial co-cultivation on amylase production of free and nano, the tested fungus *T. harzianum* and the bacterium *P. fluorescence* were grown alone (control) or as a mixed culture with different ratios from 7 days old cultures in fermentation media containing equal mixed wastes of maize.

UV visible spectral analysis Cu-chitosan NPs

Atomic absorption spectroscopy is one of the most widely used methods for quantitative analysis of various elements (Karpiuk et al. 2016). It has been used to measure the release profile or encapsulation efficacy of metals in chitosan NPs. This method is principally based on the excitation of samples by radiation and the reading of the spectra produced by it. The bio reduction of Cu-chitosan NPs in suspension was observed by ultraviolet-visible spectroscopy (UV-Vis) of the solution between 200 and 500 nm by using a Perkin-Elmer LAMBDA 35 UV-Vis spectrophotometer (USA).

Transmission electron microscope

TEM is an excellent tool for the detection of internal structure and the size of Cu chitosan NPs. The beam of electron is u Ram sea and interacts with the sample to form an image on a photographic plate. Measurements were carried out using the drop coating method in which a drop of solution containing nanoparticles was placed on the carbon-coated copper grids and kept under vacuum desiccation till dryness. TEM and high-resolution (HR)-TEM micrographs of the sample were taken using the JEM-2100F TEM instrument. The instrument was operated at an accelerating voltage of 200 kV (Ram et al. 2017).

Field experiment

Evaluating of grains dressing with fermented maize wastes by co-cultivation of antagonistic microorganisms *P. fluorescence* and *T. harzianum* against maize diseases under natural field conditions was carried out at the Experimental and Production Station, National Research Centre, Beheira Governorate, Egypt, during the summer growing season (May-September) 2018. Maize grains (*cv.* M84) were surface disinfected by immersing in sodium hypochlorite (2%) for 2 min, and washed several times with sterilized water, then air dried. Sterilized grains (at the ratio of 200 g/l) were imbibed in each of previously fermented maize wastes media for 12 h (Jensen et al. 2004). The treated grains were then air-dried and packed into plastic bags and transferred to the field for sowing. Grain dressing was carried out by applying the tested fermented maize wastes to the gum moistened grains in polyethylene bags and shaking well to ensure even distribution of the added materials. The used fermented maize wastes were those co-cultivated with *P. fluorescence* and *T. harzianum* at ratios 6:4, 7:3, and 8:2, in respective order, either supplemented or free of Cu-chitosan NPs. The treated seeds were then left on a plastic tray to air dried. The fungicide Rizolex-T50% WP at the recommended dose (3 g/kg) as well as chitosan (2%) (Sigma Aldrich Spruce Street, St. Louis, USA.) was applied as the seed dressing as stated before. In addition, disinfected, untreated maize grains were sown as a comparative treatment. A field experiment consisted of (3.5×6.0 m) plots, composed of 12 rows and a 25 cm spacing between plants within a row was established. Three replicates (plots) per each relevant treatment were used in a completely randomized block design. Two maize grains per hole were used in all the treatments. All plots received the usual agricultural practices, i.e., urea, super phosphate, and potassium nitrate (NPK) fertilizer and irrigation. Percentage of occurred diseases, i.e., root rot, damping-off (*Rhizoctonia solani*, *Fusarium* spp.), stalk rot (*Erwinia carotovora*), gray leaf spot (*Cercospora zeae-maydis*), late wilt (*Cephalosporium maydis*), and ear rot (*Fusarium verticillioides*, *Fusarium graminearum*) were

monitored and recorded throughout the growth stages from sowing up to 70 days, the experimental period.

Statistical analysis

Obtained data were subjected to the IBM SPSS software version 14.0. Analysis of variance was determined and the mean values were compared by Duncan's multiple range test at $P < 0.05$.

Results and discussion

Laboratory tests

Comparison of amylase production of free and nano by co-cultivations of *P. fluorescence* and *T. harzianum* on maize waste

Results presented in Fig. 1 shows that amylase activity increased till reached 8:2 ratios between *P. fluorescens*:*T. harzianum* produce 22.47 U/ml for free and 28.60 U/ml for nano, followed by 7:3 *P. fluorescens*:*T. harzianum* for free and nano produce 20.60 and 25.10 U/ml, respectively, then activity decreased. This result was coincided with (Vigneshwari et al. 2017) who reported that co-culturing instead of growth of one microorganism existing in the medium enhanced the activity of another strain. Co-culture of *Clostridium thermos hydrosulfuricum* and *C. thermos sulfurogenes* form complete degradation of starch, in contrast of the single cultures, the starch metabolism was not obvious in one of the strains. The same results obtained by (Fossi et al. 2014) who stated that co-culturing of *S. cerevisiae* and *Bacillus amyloliquefaciens* 04BBA15 increased amylase production. Also, Sattar et al. (2004) found that mixed culture of *A. niger* + *A. fumigatus* and mixed culture of *Thermoascus aurantiacus* and *A. niger* were suitable for amylase production. It was reported that using Cu-Chitosan nanoparticles were more effective in enzyme production by microorganisms (Gomes and Paschoalin, 2017)The

fungal strain *A. fumigatus* TFR-8 was able to turn the metal salt of macro and micro scale to nano-scale diameter by extracellular catalytic enzyme effect. This process is known as biosynthesis of metal nanoparticle. The biological structure of nanoparticles is characterized as safe and cheap because they are covering agent with naturally fungal protein (Ramesh and Tarafdar 2013). Co culturing of *T. harzianum* and *C. globosum* was the most promising in enzyme production (El Gamal et al. 2018).

UV visible spectral analysis Cu-chitosan NPs

The results illustrated in (Fig. 2) showed that the wavelength ranged at 220 nm. Each atom of metals absorbs a certain light wavelength converted to excited states. Since each atom or element has different degrees of refraction according to wavelength, therefore, the produced energy is measured as a form of photons of light in the tested samples (Jaiswal et al. 2012). Cu encapsulation was added from 70 to 80%. This might be due to permeable structure of chitosan NPs (Saharan et al. 2013). The granule and spherical morphology of nanoparticles from *B. subtilis* and *P. fluorescens* were presented at the particle sizes ranged from 100-200 nm (El-Mohamedy et al. 2018).

Transmission electron microscopy

The results are presented in (Fig. 3) revealed that round and the granule shape morphology of Cu-chitosan NPs was required at 0.5 μm . The results agree with Brunel et al. (2013) who stated that Cu-loaded chitosan NPs have spherical constriction with size between 100 up to 500 μm . Also, the nanoparticles size of Cu-chitosan was in the range of 200–600 nm as reported by Saharan et al. (2013). While Liu and Gao (2009) recorded a smooth exterior shape of chitosan NPs with a size ranged between 150 up to 350 nm.

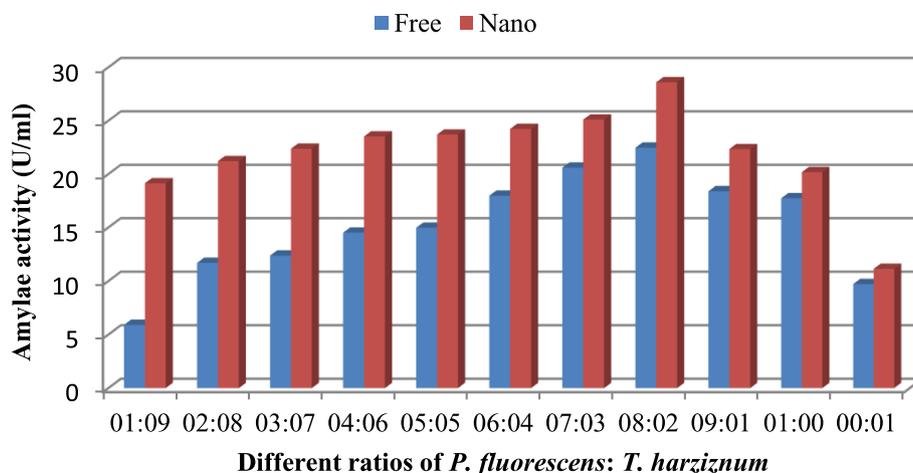


Fig. 1 Effect of different ratios of *T. harzianum* and *P. fluorescens* maize waste

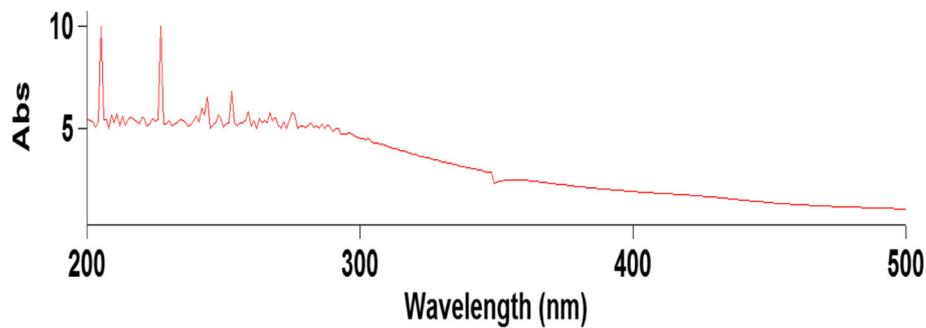


Fig. 2 UV visible spectral analysis Cu-chitosan NPs

Field experiment

Under field conditions, grain dressing with fermented maize wastes by Co-cultivation of antagonistic microorganisms, *T. harzianum* and *P. fluorescence* against maize disease incidence was evaluated. As shown in (Table 1 and Fig 4) applied treatments could significantly reduce both root and foliar diseases of maize plants than the untreated check control. Also, treated grains with fermented maize wastes supplemented with Cu-chitosan (CNPs) had a superior effect on the incidence of diseases compared to grain dressing with fermented maize wastes free of Cu-chitosan (FNPs) treatment. Also, those treatments had a higher effect on disease incidence that those of chitosan and fungicide treatments. Data also shows that grain dressed with CNPs at the ratios of 8:2, 7:3, and 6:4 by co-cultivation antagonists, *P. fluorescence* and *T. harzianum* revealed the lowest root rot and damping-off incidence recorded as 1.2, 1.4, 1.7%, followed by 1.8, 2.1, 2.2% at the same ratios of FNPs with reduction calculated as 85.8, 83.5, 80.0% and 78.8, 75.2, 74.1%, in respective relevant treatment (Fig. 1). Meanwhile, chitosan and the fungicide revealed 2.4 and 3.2% disease incidence with reduction calculated as 62.3 and 64.7, respectively compared to 8.5% of undressed grains treatment. A similar trend was also observed concerning surveyed foliar diseases. Root rot disease incidence was

recorded, in means, as 2.7, 4.3% of NPs and FNPs compared to high incidence 5.3, 6.3% at chitosan and the fungicide and 8.6% in the control treatment. Also, gray leaf spot showed, in means, 8.1 and 9.9% at NPs and FNPs which were lesser than 11.5, 12.8% at chitosan, the fungicide, respectively and 12.8% in the control treatment. Illustrated data in (Fig. 4) showed high reduction in late wilt incidence calculated as 50.0, 44.9%, followed by 33.3, 22.4% at 2 treatments of NPs and FNPs, respectively compared to low reduction 10.1% at chitosan and the fungicide over control treatment. Meanwhile, no late wilt disease reduction was recorded in the fungicide treatment. Similar studies were conducted with the role of hydrolytic enzymes produced by microorganisms and its activity against the plant pathogen or in plant self-defense. In this regards, the potency of *T. viride* and *Pseudomonas* species to control maize stalk rots diseases were recorded (Chen et al. 1999). *Trichoderma* spp. are the most common bio fungicides to control plant diseases and symbiosis with plants (Lorito et al. 2006). Chitinase and chitinolytic enzymes produced by *Trichoderma* spp. produced chitinase, which could be used as a bio control agent against plant pathogenic fungi (Kubicek et al., 2001). Likewise, *Pseudomonas cepacea* applied as a seed coating considered a biocontrol agent that could reduce the infection induced by *Fusarium*

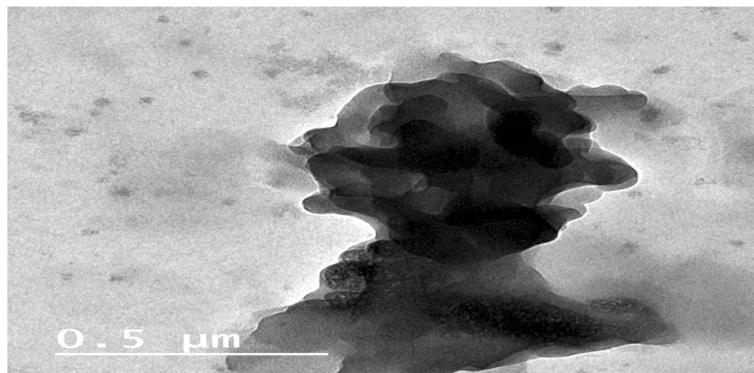


Fig. 3 Transmission electron microscopy (TEM) for spherical and granule morphology of zinc nanoparticles

Table 1 Efficacy of using fermented maize wastes as grain dressing against maize diseases under field conditions

Treatments		Root rot and damping-off ^(b)	Surveyed diseases (%) ^(c)			
			Stalk rot	Gray leaf spot	Late wilt	Ear rot
Cu- chitosan NPs	6 Ps + 4 _{Tr} (a)	1.7 ± 0.3 ^(d) d	3.0 ± 0.3 e	9.2 ± 0.4 d	7.6 ± 0.3 f	6.9 ± 0.3 g
	7 Ps + 3 Tr	1.4 ± 0.2 d	3.0 ± 0.2e	8.4 ± 0.2 e	7.6 ± 0.2 f	8.5 ± 0.7 h
	8 Ps + 2 Tr	1.2 ± 0.2d	2.3 ± 0.1f	6.9 ± 0.3 f	6.9 ± 0.3 g	7.6 ± 0.2 i
	Mean	1.4	2.7	8.1	7.3	8.5
Free of NPs	6 Ps + 4 Tr	2.2 ± 0.4 c	4.6 ± 0.2 d	10.7 ± 1.0 c	11.5 ± 0.7 d	12.0 ± 0.4 d
	7 Ps + 3 Tr	2.1 ± 0.3 c	4.6 ± 0.2 d	10.0 ± 0.8 c	10.7 ± 0.4 d	11.3 ± 0.6 e
	8 Ps + 2 Tr	1.8 ± 0.4 d	3.8 ± 0.2 e	9.2 ± 0.4 d	9.2 ± 0.2 e	10.7 ± 0.9 f
	Mean	2.0	4.3	9.9	10.4	11.3
Chitosan		2.4 ± 1.2 c	5.3 ± 0.2 c	11.5 ± 0.7 b	12.3 ± 0.1 c	13.8 ± 0.6 c
Rizolex-50 WP		3.2 ± 0.4 b	6.3 ± 0.1 b	12.8 ± 0.4 a	12.2 ± 0.4 b	14.6 ± 0.4 b
Control		8.5 ± 0.3 a	8.6 ± 0.5 a	12.8 ± 1.2 a	13.8 ± 0.2 a	15.3 ± 0.3 a

^(a)Ps *Pseudomonas fluorescence*, Tr *Trichoderma harzianum*

^(b)Mean values of root-rot and damping-off diseases incidence calculated up to 30 days of sowing as follows: *disease incidence* no. of infected plants/no. of sown grains X 100

^(c)The percentage of each disease incidence was calculated as follows:

disease incidence no. of infected plants/no. of total plants X 100

^(d)Means ± standard deviations within a column followed by the same letter are not significantly different by Duncan multiple range test at $P < 0.05$

moniliforme of maize roots by 23–80% (Hebbar et al., 1992a, b). A range of soil-borne plant pathogenic fungi including *F. graminearum*, *F. moniliforme*, and *Macrophomina phaseolina* were inhibited by *P. cepacia*.

Recently, in order to avoid human health and environment harmful effect, the use of fungicide alternatives had growing regard. The regular examination of the interaction between microorganisms and plant products is considered one of the major processes utilized for detecting biologically active substances. Further, antibiotics, alkaloids, terpenes, cyanogenic glucosides, chitinases, beta-

1,3-glucanases, lectins, arcelins, vicilins, systemins, amylase, and enzyme inhibitors areaproteic or proteic compounds were found to be produced by evolving plants, which have a certain degree of resistance. In this regards, Amylases (alpha-amylase, beta-amylase) are a group of enzymes produced by different bacteria and fungi (Taniguchi and Honnda, 2009). The two enzymes α -amylase and glucoamylase were produced by several fungi and bacterial strains in solid state fermentation system using wheat bran, cassava flour, sugar cane bagasse, rice straw, corncob, and crushed corncob as carbon sources of these

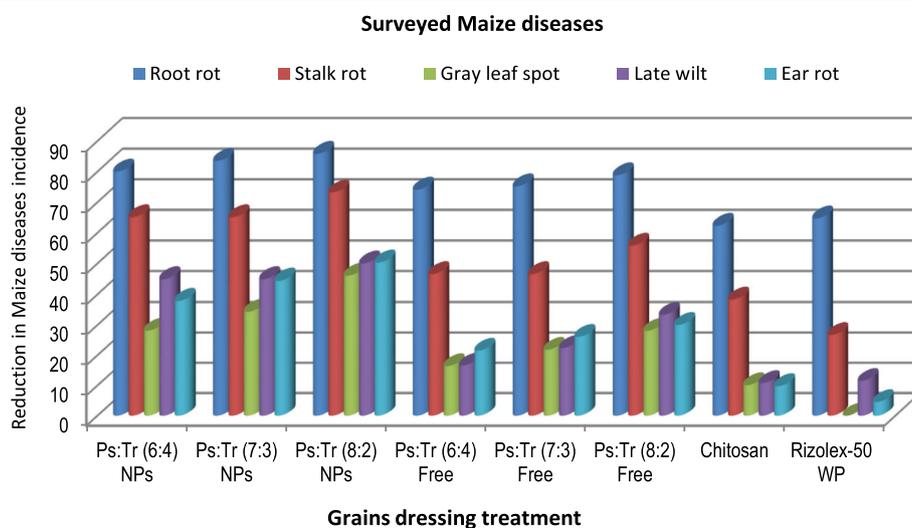


Fig. 4 Reduction in Maize diseases in response to using fermented maize wastes as grain dressing under field conditions

agricultural residues under in vitro conditions (Peixoto-Nogueira et al. 2008). The produced enzyme and by *Rhizopus* microspores isolated from seed germination test of maize were determined by (Kapilan and Anpalagan 2015). These antifungal and antibacterial efficacy tested against various fungal and bacterial strains, e.g., *Pythiummultinum*, *Rhizoctonia solani*, *Fusarium oxysporium*, *Bacillus* sp., *Escherichia coli*, *Pseudomonas*, and *Streptococcus*. Also, Sing and Kaur (2015) stated that ethanol extract of *Phyllanthus emblica* leaves have effective amylase inhibitory potential. It contains phenols and citric acid that is important for health as antioxidants. They added that this extract showed antibacterial effectiveness of the extract against pathogenic bacterial strains *Escherichia coli* and *Staphylococcus aureus* test organisms.

Moreover, the produced enzymes α -amylases by plants are used as defense mechanism tools against insect pests (Kluh et al. 2005). Also, amylase was reported to have inhibitor action against insect larvae, and it can be used as an insecticidal protein to prevent the growth of insect larvae that infest seed. Therefore, the use of insecticidal protein has the beneficial capability for agricultural crop production as well as the environment and consumers through the reduced use of chemical pesticides and insecticides (Farias et al. 2007). The cumulative knowledge revealed that one of the most efficient means to mobilize plant defensive properties are the enzymes and their inhibitors, which related to exogenous hydrolytic enzymes by specific inhibitors. Hydrolytic enzymes, such as proteases and carbohydrases (pectinases, cellulases, and amylases) excreted by plant pathogens are one of the earliest aspects of the pathogen to attack plant tissues. The role of amylases depends on splitting starch, a carbohydrate stock in plants. Induction of the structure of proteinaceous inhibitors, which suppress the efficacy of alien enzymes is a result of hydrolases acted by the pathogen on plants. Pathogenesis is determined by the interactions between the two partners, the host plant and the pathogen, therefore, the involvement of “hydrolase–inhibitor” complex should be considered for each particular path system (El-Gamal et al. 2017).

Conclusion

The study focused on finding biocontrol agents, as fungicide alternative control tools, against various soil-borne and airborne pathogens compound that characterized as the safe application to human and the environment. Therefore, the fermented maize wastes by co-cultivation of antagonistic microorganisms, *P. fluorescence* and *T. harzianum* against maize root and foliar disease incidence, when used as grain treatment before sowing under natural field conditions were evaluated.

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Ethical approval and consent to participate

Not applicable

Authors' contributions

Sherien Mohamed Mabrouk Atalla (SH): selection of microorganism, enzymes assay, nanotechnology tests. Nadia G. El-Gamal (NG): selection of microorganism, field experiment, nanotechnology tests. Mokhtar M. Abdel-Kader (Mk): field experiment. Nehal S. El-Mougy: field experiment. All authors read and approved the final manuscript.

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Author details

¹Chemistry of Natural and Microbial Products Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki, P.O. Box: 12622, Giza, Egypt. ²Plant Pathology Department, Agricultural and Biological Research Division, National Research Centre, Dokki, Giza 12622, Egypt.

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