

Improvement of Soil Properties, Growth of Cucumber and Protection against *Fusarium* Wilt by *Piriformospora indica* and Two Industrial Organic Wastes

Moustafa H.A. MOHARAM^{1,3*}, Mazhar D.A. MOHAMED²,
Osama E. NEGIM³

¹Sohag University, Faculty of Agriculture, Plant Pathology Department, New Campus at El-Kawamel, Sohag P.O. 82755, Egypt; moustafa.moharam@agr.sohag.edu.eg

²Sohag University, Faculty of Agriculture, Microbiology Department, New Campus at El-Kawamel, Sohag P.O. 82755, Egypt; mazhareisawi@yahoo.com

³Sohag University, Faculty of Agriculture, Soil and Water Sciences Department, New Campus at El-Kawamel, Sohag P.O. 82755, Egypt; onegim@yahoo.com (*corresponding author); moustafa.moharam@agr.sohag.edu.eg

Abstract

The current work was focused on characterizing bagasse ash (BA) and press mud (PM) as soil amendments and to study their effect in combination with the endophytic fungus *Piriformospora indica* on *Fusarium* wilt (FW) of cucumber caused by *Fusarium oxysporum* f. sp. *cucumerinum* (Fo). Whereas BA and PM improved almost all physico-chemical properties of the soil evaluated, seed treatment with *P. indica* had no such effect. In shake culture in potato dextrose broth (PDB) medium amended with aqueous extracts of BA and PM, alone or in combination, production mycelial mass of Fo was significantly decreased by PM extract, while production mycelial mass of *P. indica* was highly improved. The colonization rate of cucumber roots by *P. indica* as determined by microscopy was highly increased by increasing amounts of BA, PM and BA+PM added to the soil. Seed treatment of cucumber with *P. indica* before plant cultivation in non-amended soil significantly decreased the disease severity of FW and improved plant growth. When seed treated with *P. indica* was sown into soil amended with BA, PM or the combination of both, the disease severity was even more reduced than after seed treatment with *P. indica* alone. In this respect, amendment with PM was more effective than with BA, and the combinations were more effective than the single treatments. Hence, there is a scope to integrate PM and BA as soil amendments in combination with *P. indica* for eco-friendly FW management, improving soil properties and growth of cucumber plants.

Keywords: cucumber, *Fusarium* wilt, *Piriformospora indica*, organic waste, protection

Abbreviations: autoclaved soil (AS), bagasse ash (BA), barley sand medium (BSM), colony forming unit (cfu), deoxyribo nucleic acid (DNA), diethylenetriamine penta acetic acid (DEPTA), Disease severity (DS), distilled water (DW), sterile distilled water (SDW), dry weight (DrW). electrical conductivity (ECe), fresh weight (FrW), *Fusarium oxysporum* (Fo), *Fusarium* wilt (FW), non-autoclaved soil (NAS), Nutrient agar (NA), polymerase chain reaction (PCR), potato dextrose agar (PDA), press mud (PM), organic matter (OM). *Piriformospora indica* (*P. indica*).

Introduction

Because of the fact that soils recently reclaimed in the arid and semi-arid areas of Upper Egypt are very poor in organic matter, total N, available P, K and other essential macronutrients and micronutrients such as Fe, Cu, Mn and Zn, it is highly desirable to improve the physical and

chemical soil properties for the highest possible productivity of crops. For improving soil fertility in these areas, most farmers commonly tend to use unsafe muddy soils and organic materials that are often contaminated with diseases and chemicals causing harmful effects on plant, animal, and human. Therefore, alternative, safe ways to improve soil properties, crop productivity, and disease resistance are urgently needed. In this context, seed treatment with

microorganisms along with the use of available safe organic soil amendments such as compost, vegetable residues, animal manures and organic wastes may be an alternative strategy.

For this purpose, various organic wastes of plant materials originating from controlled industrial sources have been reported as soil organic amendments for altering the physical, chemical and biotic conditions of the soil, improving plant growth and production (Razzaq, 2001; Solaimalai et al., 2001; Pandya et al., 2009; Sarwar et al., 2010; Khan, 2011; Ghulam et al., 2012; Patil et al., 2013; Yadav, 2014; Dotaniya et al., 2016; Nigem et al., 2016) and reducing the inoculum potential of pathogens (Pandya et al., 2009; Yadav, 2014; Dotaniya et al., 2016; Gilardi et al., 2016). Of such organic wastes available in Egypt, bagasse ash (BA) and press mud (PM) are industrial wastes associated with sugar production from sugarcane. The annual amount of these materials in sugar factories have been estimated at about 36 and 316 thousand tons of BA and PM, respectively, that have to be disposed of in an environmentally safe way (Nakhla and El Haggag, 2014). Although, sugarcane BA and PM are considered pollutants, they contain various nutrients that are vital for improving soil fertility, plant growth and production (Solaimalai et al., 2001; Rangaraj et al., 2007; Khan, 2011; Dotaniya et al., 2016; Moharam et al., 2016; Nigem et al., 2016). In general, BA has 47-52% hemicellulose, 25-28% cellulose, and 20-21% lignin sugars, whereas PM contains 50-70% moisture, 20-30% fiber, 7-15% crude wax, 5-12% sugars, 5-10% crude protein and 2-2.5% nitrogen, and significant amounts of Fe, Mn, Ca, Mg, Si, K, and P (Solaimalai et al., 2001; Gupta et al., 2011; Khan, 2011; Dotaniya et al., 2016). Considering the richness of nutrients of sugarcane PM and the mobilization in soil of insoluble P by organic acids produced from BA (Dotaniya, 2014; Dotaniya and Datta 2014; Dotaniya et al., 2016), both have the potential of becoming useful products for use in agriculture (Solaimalai et al., 2001). Based on several studies carried out in pots or under field conditions, application of BA or PM for improving soil physical, chemical and biological properties, and increasing organic matter and nutrient content has been recommended (Bokhtiar et al., 2000; Rangaraj et al., 2007; Jamil et al., 2008; Singh et al., 2008; Sarwar et al., 2010; Khan, 2011; Dotaniya et al., 2016).

The cucumber plant (*Cucumis sativus* L.) is one of the main economic vital vegetable crops grown in fields and under protected conditions. In Egypt and worldwide, the major disease causing severe losses in cucumber production is FW caused by the form *specialis* (f.sp.) *cucumerinum* of the soil-inhabiting fungus *Fusarium oxysporum* Schlecht. Fr. (Jun-Li et al., 2010; Moharam and Negim, 2012). Several practices such as use of the resistant or tolerant cultivars, crop rotation, and fumigation to decrease the damage of FW have been suggested before (Yu, 2001). Also, fungicide application is common, but may not be effective especially when the wilt appears late in the season (Srivastava et al., 2010).

Seed treatment with systemic fungicides before sowing controls the disease effectively, however, there are strong objections against their use due to potential toxic or other

harmful effects in the environment. Hence, use of effective microorganisms along with organic wastes for FW management may be an alternative eco-friendly option.

Previous studies have shown the potential of certain bacteria and fungi to control FW, increase uptake of minerals and promote plant growth (Kennedy and Smith, 1995; Doran et al., 1996; Shivanna et al., 1996; Moharam and Negim, 2012). One of the candidate fungi recently tested for these purposes is *P. indica*, a plant-root-colonizing fungus belonging to the Basidiomycotina. It is a plant growth promoter which colonizes the root cortex and hairs of various plants and promotes nutrient and water uptake especially under extreme environmental stresses (Waller et al., 2005; Oelmüller et al., 2009; Zuccaro et al., 2009; Varma et al., 2012a; Varma et al., 2013; Bagheri et al., 2014; Moharam et al., 2017). It was also shown to confer systemic resistance and tolerance to pathogens in various plants (Varma et al., 2012b; Johnson et al., 2014; Moharam et al., 2017).

A number of publications have recently documented the potential of *P. indica* to control root diseases caused by *Fusarium* spp., e.g. in barley against *F. culmorum* and *F. graminearum* (Waller et al., 2005; Deshmukh and Kogel, 2007; Harrach et al., 2013), in wheat against *F. oxysporum* (Moharam et al., 2017), in maize against *F. verticillioides* (Kumar et al., 2009), in tomato against *F. oxysporum* (Fakhro et al., 2010; Sarma et al., 2011) and in lentil against *F. oxysporum* f.sp. *lentis* (Dolatabadi et al., 2012). Despite the general potential of organic amendments applied alone or along with different microorganisms to control wilt diseases (Chakrabarti and Sen, 1991; Padmodaya and Reddy, 1999; Padmodaya, 2003; Bonanomi et al., 2007; Njoroge et al., 2008; Borrego-Benjumea et al., 2014; Gilardi et al., 2016), only limited information is available on the use of sugarcane PM as soil amendment in combination with microorganisms like *Trichoderma harzianum* or *Pseudomonas fluorescens* against tomato wilt disease caused by *F. oxysporum* f. sp. *lycopersici* (Yadav, 2014). Also, populations in soil of bacteria and fungi increased (Ownen, 1954; Ochoa-George et al., 2010) while nematodes (Anonymous, 1971; Alexander, 1972) and pathogenic fungi such as *Fusarium* and *Pythium* were suppressed (Anonymous, 1968). *In vitro*, aqueous extracts of PM caused 20.78% inhibition of *F. solani* growth (Pandya et al., 2009). Also, preparations from cashew (*Anacardium occidentale* L.) peduncle bagasse ash showed antifungal activity against *F. oxysporum*, *F. moniliforme* and *F. lateritium* (Santos et al., 2011). Therefore, the current study was carried out to evaluate the potential of *P. indica* and the two industrial organic wastes BA and PM applied to the soil alone or in combination for improving soil properties, growth and FW control of cucumber. Another objective of this investigation was to study the effects of BA and PM on growth of both *Fo* and *P. indica in vitro*, and on root colonization by *P. indica* in pot experiments. Further, the potential of *P. indica* to colonize the roots of cucumber plants grown in pots was studied by microscopy and PCR diagnosis.

Materials and Methods

Soil used and its properties

The soil used here was collected from the Experimental Farm, Faculty of Agriculture, Sohag University at the geographical site of El-Kawamel, Sohag Governorate of Upper Egypt. The soil was immediately sieved through a screen (4-mm mesh) for use in the experiments, and through a 2-mm mesh screen for use in analyses of physical and chemical properties before planting (Khan, 2011). A soil aliquot (50 g) was used for particle size distribution analysis by sieving and pipette methods (Richards 1954). Dry bulk density and total porosity were determined by the core method according to the methodology described by (Blake and Hartge, 1996). Soil pH was measured in 1:1 soil: water suspension by pH meter (Orion model 410A) according to Jackson (1973). Electrical conductivity was determined in soil past extract (EC_e) using electrical conductivity meter (Orion model 150) according to Jackson (1973). Organic matter (OM) content was estimated by a modified Walkley-Black method as elaborated by Jackson (1967). Calcium carbonate content was also determined volumetrically using the calibrated Collin's Calcimeter method as described by Jackson (1973). Soluble cations and anions were analysis in the saturated soil paste extracted according to Jackson (1973). Total N was determined by Kjeldahl procedure (Jackson, 1973). K was determined by ammonium acetate method (Black, 1965). P was determined by formation of a phospho molybdate complex (Lennox, 1979). Available metal content in the soil was determined by ICP mass Spectrometer (Icap6000 Series- Thermo Fisher Scientific Company) after using DTPA extractable micronutrients from the soil samples by 0.05 M DTPA at pH 7.3 according to Lindsay and Norvel (1978). The physico-chemical characteristics of this soil determined are listed in Table 1.

Organic wastes: properties, extraction and amendment application

The organic wastes BA and PM used in the present investigation were obtained from the industry sugar Mills, Qena Governorate, Egypt. The materials were ground to pass through 2 mm sieve and analysed to determine different chemical characteristics. Table 2 presented the chemical characteristics of PM and BA. To prepare the stock of 10% aqueous extract of BA and PM, 10 g of each organic material were suspended in 100 ml distilled water (DW) in conical flasks, and placed on a rotary shaker at room temperature. After 25 days, the contents of the flasks were carefully filtered through double layers of muslin cloth, autoclaved for 20 min (Pandya *et al.*, 2009) and then kept in a refrigerator for later use. Before starting the trials, the organic materials of BA, PM and BA+PM were carefully mixed into the soil (2.5 and 5%, w:w). The mixture was then irrigated with water (to reach 75% of the soil moisture capacity) and afterwards immediately covered with transparent polyethylene sheets. After 14 days, the cover was removed and the mixture was left to dry with daily mixing for 2 weeks before use.

Plant and fungal materials and inoculation methods

Cucumber seeds of cultivar Denmark Beta-Alpha, which is susceptible to FW (Moharam and Negim, 2012) were used. Seeds were surface disinfected with 1.5% sodium hypochlorite for 10 min, rinsed 3 times with sterile distilled water (SDW), and then immersed again in SDW for 3 h followed by placement in hot SDW at 60 °C for 5 min to kill any endophytes inside the seeds (Huang and Backhouse, 2005) and then left for air drying under aseptic conditions. Seeds were then tested for absence of the internal endophytes before treating and planting by placing subsamples of treated seeds in Petri plates on potato dextrose agar (PDA) medium.

Table 1. The physico-chemical characteristics of the studied soil

Properties	Unit	Soil
Texture grade		Sandy loam sand (78%), silt (9%), clay (13%)
Dry bulk density	g cm ⁻¹	1.41
Total porosity	%	52
pH		8.11
EC _e	dSm ⁻¹	1.3
Organic matter	%	0.4
CaCO ₃	%	1.1
HCO ₃ ⁻	meq/l	7.4
SO ₄ ⁻	meq/l	4.5
Cl ⁻	meq/l	2.66
Soluble Na ⁺	meq/l	2.12
Ca ⁺⁺ + Mg ⁺⁺	meq/l	9.4
Total N	mg kg ⁻¹	0.5
Available P	mg kg ⁻¹	0.3
Available K	mg kg ⁻¹	1.1
Fe	ppm	22
Zn	ppm	0.8
Cu	ppm	0.4
Mn	ppm	27

Table 2. Chemical analysis of BA and PM used in this study

Analysis	Unit	BA	PM
Form		Powder	Soft, spongy
pH		8.6	7.22
EC	dSm ⁻¹	2.38	3.44
Total N	%	0.4	0.9
Total P	%	0.3	0.8
Total K	%	0.5	0.3
Ca	%	0.4	0.9
Mg	%	0.5	0.6
OM	%	3.40	6.64
Fe	ppm	442	536
Zn	ppm	35	102
Cu	ppm	66	44
Mn	ppm	78	203

Two fungal isolates, *F. oxysporum* f. sp. *cucumerinum* (Fo) and *P. indica* used in this study were obtained from the culture collections of the Department of Plant Pathology, Sohag University, Egypt. To prepare the inoculum of Fo, the fungus was grown in autoclaved barley sand medium (BSM) at 28±5 °C for two weeks. Afterward, the inoculum was added to the BA and PM -amended soil (3%, w:w) as described above. The mixture was then slightly irrigated every other day for a week before planting. The stock culture of *P. indica* was cultured at 28±5 °C and maintained at 4 °C on slope containing Kaefer agar medium (Prasad *et al.*, 2005). To prepare inoculum of *P. indica*, the fungus was re-cultured on PDA plates at 28±5 °C and then propagated on a rotary shaker at 28±5 °C in PDA broth medium for two weeks. For inoculation with *P. indica*, cucumber seeds were immersed in an aqueous solution of 0.05% Tween 20 containing 5×10^5 chlamydospores ml⁻¹ for one hour before sowing (Varma *et al.*, 1998).

In vitro test for antagonistic activity

Antagonism between *P. indica* and Fo was tested by the dual culture technique described by Ghahfarokhi and Goltapeh (2010). A 7-mm disc of Fo mycelium taken from the peripheral region of a 7 day-old culture was placed on one side of a PDA plate (9 cm in diameter). A 7 mm disc of *P. indica* obtained from the margin of a 14 day-old culture was placed on the other side of the plate. PDA plates inoculated with Fo or *P. indica* alone served as controls. All plates were then incubated at 28±5 °C for 7 days and afterwards the radial growth (cm) of Fo and *P. indica* was measured. The entire experiment included 4 plates of each treatment and was repeated twice. Percent growth inhibition was calculated by the formula: Inhibition of mycelial growth (%) = $C-T/C \times 100$. Where C = growth in control and T = growth in treatment.

In vitro effects of aqueous extracts of organic wastes on growth of Fo and *P. indica*

The effect of aqueous extract of BA and PM on biomass production of Fo and *P. indica* was determined. The stock of 10% aqueous extract of each organic material (see above) was added to conical flasks containing sterilized PDA broth

medium to get final concentrations of 0.5, 1, 1.5, 2, 2.5 and 3%. The controls were flasks containing PDA broth medium amended with SDW. The flasks were then inoculated with 7-mm agar discs from 7 day-old cultures of each tested fungus before incubation on a rotary shaker at 28±5 °C. Four flasks were employed for each concentration. After 7 days, the biomass production of Fo and *P. indica* as dry weight (g) was determined and means were calculated.

Greenhouse experiments

Two greenhouse trials, each laid out in a randomized block design, with six replications of each treatment were conducted in the 2016 summer growing season. Autoclaved soil (AS) and non-autoclaved soil (NAS) was amended with 0, 2.5 or 5% of each organic waste alone or in combination, inoculated with Fo (3%, w/w; preparation see above) by mixing and then slightly watered daily for a week. Soils amended with the same amount of BSM and organic wastes were used as controls. Afterward, formalin-sterilized pots (30 cm) were filled with the treated AS and NAS. Seeds of cucumber were sterilized and inoculated with *P. indica* as mentioned before, and then sown at the rate of 5 seeds per each pot. Pots were then irrigated when necessary. Percent of wilted plants was determined after 6 weeks, and the plants were then individually rated for severity of FW using a scale of 0-3, where 0 = healthy plants, no visible symptoms; 1 = weakly infected plant showing vascular discoloration, but no leaf yellowing; 2 = moderately infected plants showing leaf yellowing and wilt; 3 = severely infected plants showing plant death (Ha *et al.*, 2008). Disease severity (DS %) for each replication of each treatment was calculated by the formula: $(\sum Si \times Ni) \times 100 / (3 \times Nt)$, where Si is the severity ratings 0-3, Ni is the number of plants in each rating, and Nt is the total number of rated plants (Moharam and Negim, 2012). At 14 and 28 days after planting the roots from 10 plants per treatment were collected for light microscopy and PCR diagnosis of root colonization by *P. indica* (see below).

Six whole plants (including the root rhizosphere) were randomly taken from each treatment after 6 weeks of growth and the fresh and dry weights (g) of vegetative growth were determined. Also, total cfu-counts of Fo, *P. indica*, bacteria and other fungi in the rhizosphere per plant

were determined on PDA and Nutrient agar (NA) medium for fungi and bacteria, respectively using serial dilution method described by Vieira and Nahas (2005). Moreover, soil from each replication of each treatment was collected, homogeneously mixed and then used in analyses of physico-chemical characteristics.

Colonization of cucumber roots by *P. indica*

A- Staining and microscopy

Root samples of 14 and 28 day-old plants collected from pots were washed thoroughly with tap water. Hand sections were prepared from roots using a razor blade and stained with 0.1% trypan blue according to the method described by Kollmorgen and Ballinger (1987). Sections were then mounted in lactophenol and examined by a light microscope (Leica ATC 2000, India) equipped with a digital camera (Optika 9.2 MP, Type 4083.B9, Italy) using the 40× lens.

B- PCR diagnosis

Genomic DNA was extracted from fungal mycelium of *P. indica* and from fresh root samples of 14 and 28 day-old plants using the DNeasy Plant mini Kit (Qiagen, Germany) according to the manufacturer's instructions. Using PCR diagnosis an approx. 751-bp fragment was amplified from extracted *P. indica* DNA from both fungal pure culture (positive control) and from inoculated plants using the specific primer pair 5'-TTCTGGGAAGTCGTCTCTG-3' and 5'-AGCCAACCATGAAGAAGTG-3' targeting the annotated sequence (AJ459235) of the β -tubulin (Serfling et al., 2007). PCR reactions were performed in a total volume of 20 μ l including 0.25 μ M of each primer, 2.5 mM MgCl₂, 0.5 mM dNTPs, 1x reaction buffer, Taq DNA polymerase at 0.4 U/ μ l (all reagents from Qiagen, Germany)

and 100 ng of template DNA. Amplification reactions were performed according to a PCR protocol consisting of 95 °C for 180 s; followed by 35 cycles of 95 °C for 30 s, 51.5 °C for 30 s, and 72 °C for 90 s; and a final extension at 72 °C for 10 min. PCR products were analyzed and examined on 1% agarose gels run at 100 V in 1x TAE buffer and gels were then stained with ethidium bromide for visualization. Negative controls used either SDW or DNA extracted from roots of non-inoculated plants.

Statistical analysis

Data obtained were inputted into MSTAT-C program. Analysis of variance was performed and the least significant difference (L.S.D.) of means was calculated at P= 0.05 and 0.01 (Gomez and Gomez 1984).

Results

Interaction of *P. indica* and *Fo* in vitro

In a dual culture test performed to identify antagonistic activity of *P. indica* against and *Fo* on PDA medium after 7 days of incubation at 28±5 °C, clear antagonism not detected (Fig. 1).

Effect of aqueous extract of BA and PM on growth of *Fo* and *P. indica*

In shake culture in PDB medium supplied with aqueous extracts of BA and PM alone or in combination (Table 3), production of *Fo* mycelial mass (dry weight) was significantly decreased by PM extract. Compared to the control, mycelial mass was reduced by 25.76% at the concentration of 2.5%. Increasing the concentration to 3% had no additional effect. On the other hand, BA extract

Table 3. Effect of aqueous extracts of BA and PM on biomass production of *Fo* and *P. indica* in vitro

Extract	Concentration (%)	Biomass production (mg)*	
		<i>Fo</i>	<i>P. indica</i>
BA	0.0	295	177
	0.5	301	187
	1.0	308	198
	1.5	315	211
	2.0	323	223
	2.5	329	235
	3.0	331	237
	0.0	295	177
PM	0.5	278	183
	1.0	257	191
	1.5	237	199
	2.0	226	217
	2.5	219	226
	3.0	217	227
	0.0	295	177
	0.5	284	191
BA+PM	1.0	272	201
	1.5	267	215
	2.0	259	227
	2.5	251	239
	3.0	249	241
	0.0	295	177
	0.5	284	191
	1.0	272	201

* Dry weight after 7 days in shake culture in PDB at 28 °C. Means of four flasks each.

L.S.D. at 0.05: Aqueous extracts (AE) = 3.23; Concentrations (C) = 2.12; AE × C = 0.243.



Fig. 1. Lack of the antagonistic effect between *P. indica* and *Fo* *in vitro*. Left PDA plate (control) inoculated with two discs of *Fo*; The right plate was included with one disc of *Pi* (left) and one of *Fo* (right)

promoted the growth of *Fo*. In the case of *P. indica*, production of mycelial mass was increased by increasing concentrations of BA extract, PM extract and the combination of both. The highest production of mycelial mass was recorded for PDB amended with both BA and PM extract.

Colonization of cucumber roots by *P. indica*

Microscopic examination of hand-cut sections of cucumber roots of 14 and 28 day-old plants grown from *P. indica*-inoculated seeds and stained with trypan blue revealed the presence of *P. indica* chlamydospores within the root cortex cells (Fig. 2). At the same growth stages, *P. indica* was detected by PCR in DNA extracted from roots of plants from the same treatments. PCR amplified a single product of approx. 751-bp in length both in DNA extracted from a pure culture of *P. indica* (Fig. 3, lane 2) and in DNA extracted from the plants (lanes 4 and 5). No amplification product was detected after PCR with DNA extracted from the roots of non-inoculated plants (fig. 3, lane 3) and in the negative control (SDW, lane 1).

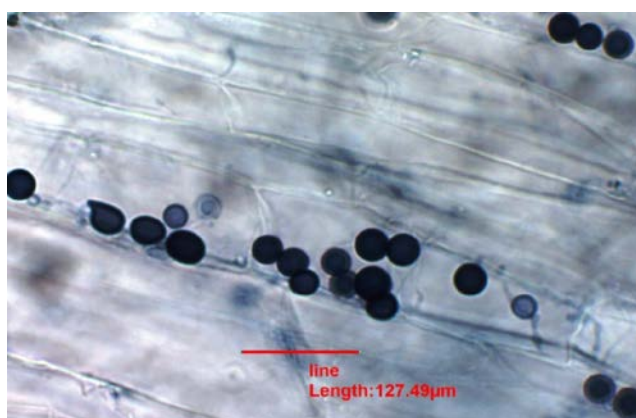


Fig. 2. A hand-cut section of cucumber roots stained with trypan blue showing the intracellular chlamydospores of *P. indica* within root cortex cells of 14 day-old plants. Likewise, in 28 day-old plants tested

Effect of organic wastes as soil amendments and seed treatment with *P. indica* on:

A- Soil physico-chemical and biological properties

A-1- Physico-chemical properties

The comparison between the soil in Table 1 and soil of the column "No amendment" in Table 4 after analysis shows that seed treatment with *P. indica* had no effect on the physico-chemical soil properties. On the other hand, amendments with BA and PM were effectively improved almost all physico-chemical properties of the soil. The degree of improvement was generally higher at the concentration of 5.0 % compared to 2.5 %. Values of dry bulk density of soil were decreased after application of BA, PM and BA+PM, compared with initial bulk density (1.4 g/cm³). It was noticed that the lowest bulk density value (1.35 g/cm³) was recorded with high rates of PM additions which ultimately led to an increase of soil total porosity as compared with the controls (no amendment). Application of PM and BA+PM also decreased the soil pH slightly,



Fig. 3. PCR detection of *P. indica* in DNA of cucumber roots with the designed specific primer pair of an annotated sequence of the β -tubulin gene. 'M' denotes a 100 bp ladder DNA size standard (AppliChem, Darmstadt, Germany). Lanes 1-5 show PCR results from different DNA templates, under otherwise identical conditions. Lane 1: sterile distilled water (negative control); Lane 2: DNA extracted from pure culture of *P. indica* (positive control); lane 3: DNA from root of non-inoculated cucumber; lane 4 and 5: DNA from the root of inoculated plants harvested after 14 and 28 days, respectively

Table 4. Effect of amendment with BA and PM and seed treatment with *P. indica* on physical and chemical properties of the soil. All treatments include a seed treatment with *P. indica*

Properties	Unit	No amendment	Amendment					
			BA		PM		BA+PM	
			2.5%	5%	2.5%	5%	2.5%	5%
Texture	-	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Dry bulk density	g cm ⁻¹	1.41	1.38	1.37	1.38	1.35	1.37	1.36
Total porosity	%	52	52.4	52.7	52.2	52.4	52.3	52.6
pH	-	8.1	8.2	8.4	7.8	7.6	8.0	7.8
EC _e	dSm ⁻¹	1.3	1.6	2.2	1.8	2.4	1.9	2.5
Organic matter	%	0.5	0.6	0.8	0.92	1.1	0.67	0.95
HCO ₃ ⁻	meq/l	7.4	7.9	9.2	8.8	10.5	8.1	10.2
SO ₄ ⁻	meq/l	4.5	5.2	7.8	6.1	8.3	7.6	8.2
Cl ⁻	meq/l	2.66	2.8	3.5	3.2	4.4	2.9	5.1
Soluble Na ⁺	meq/l	2.12	3.3	5.4	4.7	6.4	3.1	5.7
Ca ⁺⁺ + Mg ⁺⁺	meq/l	9.4	11.2	12.6	13.4	16.5	13.1	18.2
Total N	mg kg ⁻¹	0.5	0.6	0.7	0.9	1.2	0.8	1.0
Available P	mg kg ⁻¹	0.3	0.6	0.8	1.1	1.8	0.7	1.5
Available K	mg kg ⁻¹	1.1	1.6	1.8	1.8	2.3	1.5	2.4
Fe	ppm	22	28.4	32.3	30.8	36.0	27.0	33
Zn	ppm	0.8	1.2	1.8	2.6	3.9	1.8	2.7
Cu	ppm	0.4	0.9	1.1	1.2	1.6	0.9	1.3
Mn	ppm	27	28	31	32	37	29	39

whereas the BA application slightly increased the pH compared with the control. The lowest value of soil pH was recorded in the treatment receiving the high addition rate of PM and the highest value was recorded in control. Moreover, soil electrical conductivity was increased by addition of PM, BA and BA+PM compared with control. The highest value was recorded in the treatment received the maximum application rate of BA. Organic matter content of the soil was sufficiently increased by PM and BA+PM applications up to 5.0%, and the vice versa was in case BA application. It was slightly increased (0.8%) by 5.0%. Soil macro and micronutrient such as NPK, Fe, Zn, Cu and Mn were increased after application of BA, PM and BA+PM. However, application of PM (2.5%) was better than BA application (5%) for increasing soil contents of macro and micronutrients. Application of BA+PM up to 5% resulted in the highest contents of soil macro and micronutrients (Table 4) when compared to the control.

A-2- Biological properties

In general, application of PM, BA and BA+PM to the soil increased bacterial and fungal populations in the rhizosphere of cucumber plants inoculated and non-inoculated with *P. indica* as compared with non-amended soil (Table 5). Again, PM application was more effective than application of BA, application at 5% was more effective than at 2.5% and the combinations were more effective than the single treatments.

A-3- Root colonization by *P. indica*

The effects of soil amendment with BA and PM on root colonization of cucumber after seed inoculation with *P. indica* were evaluated by light microscopy and/or PCR diagnosis. The obtained results showed that the colonization rate of cucumber roots by *P. indica* as determined by microscopy (Table 6a and b) and/or PCR (data not shown) was highly increased by increasing

Table 5. Effect of organic wastes as soil amendments on bacterial and fungal population in rhizosphere of cucumber plants grown in non-autoclaved soil after inoculation of seeds with *Pi* under greenhouse conditions

Organic waste	Conc. (%)	Total count (cfu per g of soil rhizosphere)*			
		Bacteria		Fungi	
		Inoculated with <i>P. indica</i>	Non-inoculated with <i>P. indica</i>	Inoculated with <i>P. indica</i>	Non-inoculated with <i>P. indica</i>
BA	0.0	9 × 10 ⁶	11 × 10 ⁶	29 × 10 ⁴	34 × 10 ⁴
	2.5	13 × 10 ⁶	17 × 10 ⁶	33 × 10 ⁴	37 × 10 ⁴
	5.0	19 × 10 ⁶	23 × 10 ⁶	37 × 10 ⁴	41 × 10 ⁴
PM	0.0	9 × 10 ⁶	11 × 10 ⁶	29 × 10 ⁴	29 × 10 ⁴
	2.5	15 × 10 ⁶	24 × 10 ⁶	35 × 10 ⁴	39 × 10 ⁴
	5.0	22 × 10 ⁶	35 × 10 ⁶	42 × 10 ⁴	44 × 10 ⁴
BA+PM	0.0	9 × 10 ⁶	11 × 10 ⁶	29 × 10 ⁴	29 × 10 ⁴
	2.5	17 × 10 ⁶	26 × 10 ⁶	37 × 10 ⁴	42 × 10 ⁴
	5.0	25 × 10 ⁶	39 × 10 ⁶	45 × 10 ⁴	47 × 10 ⁴

* Mean population of soil rhizosphere of 12 plants each in both experiments.

Table 6a. Colonization by *P. indica* of cucumber roots of 28 day-old plants grown in autoclaved soil without or with amendments. The table shows the number of colonized plants determined by light microscopy (n=20)

No amendment	Amendment					
	BA		PM		BA+PM	
	2.5%	5%	2.5%	5%	2.5%	5%
10 (% increase)	11 (10%)	13 (30%)	13 (30%)	15 (50%)	14 (40%)	17 (70%)

Table 6b. Colonization by *P. indica* of cucumber roots of 28 day-old plants grown in non-autoclaved soil without or with amendments. The table shows the number of colonized plants determined by light microscopy (n=20)

No amendment	Amendment					
	BA		PM		BA+PM	
	2.5%	5%	2.5%	5%	2.5%	5%
8 (% increase)	8 (0%)	10 (25%)	10 (37.5%)	13 (62.5%)	12 (50%)	13 (75%)

Table 7a. Effect of seed inoculation of cucumber with *P. indica* and soil amendment with organic wastes on disease severity of FW in the greenhouse in autoclaved soil

Inoculation	No amendment	Amendment								
		BA			PM			BA+PM		
		2.5%	5%	Mean	2.5%	5%	Mean	2.5%	5%	Mean
Fo	39.7***	38.6 ^a	37.4 ^a	38.0 ^a	36.8 ^a	34.7 ^a	35.8 ^a	37.1 ^a	33.7 ^a	35.4 ^a
Fo+ <i>P. indica</i>	14.6 ^b	2.5 ^b	1.3 ^b	1.9 ^b	1.2 ^b	0.7 ^b	0.9 ^b	0.4 ^b	0.1 ^b	0.2 ^b
Control*	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c
L.S.D. 0.05	0.15	0.09	0.04	0.08	0.07	0.04	0.04	0.04	0.04	0.04

* No inoculation with Fo and *P. indica*.

** The values are means of disease severity (%) from total 60 plants represent each treatment in both experiments. Letters a, b, c and d of values are the rank of significant difference according to ANOVA (L.S.D. p = 0.05).

Table 7b. Effect of seed inoculation of cucumber with *P. indica* and soil amendment with organic wastes on disease severity of FW in the greenhouse in non-autoclaved soil

Inoculation	No amendment	Amendment								
		BA			PM			BA+PM		
		2.5%	5%	Mean	2.5%	5%	Mean	2.5%	5%	Mean
Fo	36.7***	34.6 ^a	32.4 ^a	33.5 ^a	32.4 ^a	30.6 ^a	31.5 ^a	30.7 ^a	28.4 ^a	29.6 ^a
Fo+ <i>P. indica</i>	11.2 ^b	2.1 ^b	1.3 ^b	1.7 ^b	0.8 ^b	0.3 ^b	0.5 ^b	1.1 ^b	0.4 ^b	0.7 ^b
Control*	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^c
L.S.D. 0.05	0.13	0.07	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04

* No inoculation with Fo and *P. indica*.

** The values are means of disease severity (%) from total 60 plants represent each treatment in both experiments. Letters a, b, c and d of values are the rank of significant difference according to ANOVA (L.S.D. p = 0.05).

amounts of BA, PM and BA+PM added to the soil, PM application was more efficient than BA application, however, application of BA+PM caused the highest root colonization by *P. indica* 70 and 75% in both autoclaved and non-autoclaved soil treated, respectively.

B- FW control and growth of cucumber

B-1- 1- FW control

It is clear from Table 7 a and b that amendment of the soil with BA or PM had no or just a slight effect on Fo, both

in autoclaved and non-autoclaved soil. Seed treatment with *P. indica* before cultivation in non-amended soils, autoclaved or non-autoclaved, significantly decreased the disease severity from 39.7 and 36.7% to 14.6 and 11.2%, respectively. When seed treated with *P. indica* was sown into soil amended with BA, PM or the combination of both, the disease severity was even more reduced than after seed treatment with *P. indica* alone. In this respect, amendment with PM was more effective than with BA, and the combinations were more effective than the single treatments.

Table 8. Effect of soil amendment with organic wastes on populations of Fo and *P. indica* in the rhizosphere of cucumber plants in autoclaved and non-autoclaved soil

Amendment	Conc. (%)	Total count (cfu × 10 ⁴ per g of soil rhizosphere)*			
		Fo		<i>P. indica</i>	
		Autoclaved	Non-autoclaved	Autoclaved	Non-autoclaved
BA	2.5	32	19	14	11
	5.0	27	15	19	15
PM	2.5	23	17	17	13
	5.0	18	11	21	18
BA+PM	2.5	19	15	19	16
	5.0	13	9	23	21
No amendment	0.0	43	32	9	7

* Means of 6 plants per treatment each from two separate experiments.

Table 9a. Effect of seed inoculation of cucumber with *P. indica* and soil amendment with organic wastes on fresh weight (FrW) and dry weight (DrW) per plant six weeks after sowing in autoclaved soil

Inoculation	No amendment		Amendment											
			BA				PM				BA+PM			
			2.5%		5%		2.5%		5%		2.5%		5%	
	FrW	DW	FrW	DrW	FrW	DrW	FrW	DrW	FrW	DrW	FrW	DrW	FrW	DrW
Fo	134.6** ^a	9.6 ^a	138.2 ^a	11.2 ^a	143.4 ^a	11.5 ^a	151.4 ^a	11.8 ^a	156.2 ^a	12.8 ^a	158.6 ^a	12.8 ^a	177.4 ^a	14.6 ^a
<i>P. indica</i>	287.2 ^b	23.4 ^b	374.2 ^b	31.6 ^b	402.2 ^b	34.4 ^b	415.6 ^b	33.8 ^b	476.4 ^b	38.8 ^b	437.4 ^b	35.7 ^b	489.6 ^b	39.8 ^b
Fo+ <i>P. indica</i>	211.4 ^c	18.6 ^c	267.4 ^c	23.6 ^c	286.6 ^c	25.2 ^c	306.5 ^c	28.2 ^c	354.4 ^c	31.8 ^c	298.2 ^c	27.4 ^c	387.4 ^c	34.2 ^c
Control*	237.6 ^d	21.4 ^d	252.2 ^d	22.2 ^d	254.2 ^d	23.6 ^d	264.6 ^d	25.4 ^d	282.4 ^d	26.8 ^d	271.4 ^d	25.2 ^d	305.4 ^d	28.2 ^d

* No inoculation with Fo and *P. indica*.

** Means of 6 plants per treatment each from two separate experiments. Letters a, b, c and d of values are the rank of significant difference according to ANOVA (L.S.D. p = 0.05).

Table 9b. Effect of seed inoculation of cucumber with *P. indica* and soil amendment with organic wastes on fresh weight (FrW) and dry weight (DrW) per plant six weeks after sowing (non-autoclaved soil)

Inoculation	No amendment		Amendment											
			BA				PM				BA+PM			
			2.5%		5%		2.5%		5%		2.5%		5%	
	FrW	DW	FrW	DrW	FrW	DrW	FrW	DrW	FrW	DrW	FrW	DrW	FrW	DrW
Fo	151.4** ^a	12.2 ^a	156.4 ^a	12.2 ^a	163.2 ^a	12.8 ^a	161.4 ^a	12.8 ^a	177.2 ^a	14.2 ^a	166.6 ^a	13.6 ^a	181.4 ^a	15.6 ^a
<i>P. indica</i>	295.4 ^b	24.2 ^b	381.6 ^b	32.2 ^b	413.2 ^b	35.4 ^b	427.6 ^b	34.2 ^b	488.4 ^b	39.3 ^b	445.4 ^b	37.2 ^b	501.6 ^b	41.4 ^b
Fo+ <i>P. indica</i>	226.6 ^c	18.6 ^c	286.2 ^c	23.6 ^c	297.6 ^c	26.2 ^c	316.4 ^c	29.2 ^c	366.4 ^c	32.6 ^c	332.6 ^c	28.4 ^c	403.4 ^c	33.6 ^c
Control*	243.4 ^d	19.4 ^d	269.4 ^d	22.2 ^d	265.2 ^d	24.6 ^d	277.6 ^d	26.4 ^d	298.4 ^d	27.8 ^d	283.2 ^d	22.6 ^d	323.6 ^d	25.4 ^d

* No inoculation with Fo and *P. indica*.

** Means of 6 plants per treatment each from two separate experiments. Letters a, b, c and d of values are the rank of significant difference according to ANOVA (L.S.D. p = 0.05).

B-1-2- Population of Fo and *P. indica* in plant rhizosphere

Application of BA and PM alone or in a mixture led to a clear decrease of the Fo population in both autoclaved and non-autoclaved soil (Table 8). PM was more effective than BA, however, a mixture of BA+PM was even more effective. On the other hand, application of both amendments alone or in a mixture resulted in an increased *P. indica* population in the autoclaved and non-autoclaved soil. Again, PM was more effective than BA in this respect while the mixture was most effective.

B-2- Growth of cucumber

In non-amended, autoclaved (Table 9a), healthy soil (without inoculation of Fo), seed treatment with *P. indica* increased the fresh weight by around 20 %. In amended soil the increase varied between about 48 and 62%. The result obtained with non-autoclaved soil (Table 9b) was very similar, with an increase of around 21% in non-amended soil and increases between 42 and 64% in amended soil. In non-amended, autoclaved (Table 8), infected soil (= with inoculation of Fo), seed treatment with *P. indica* increased the fresh weight by 57%, and in the presence of amendments this value almost tripled to reach 170 - 205%. In non-autoclaved soil (Table 9b) seed treatment with *P. indica* resulted in an increase of around 50%. The increase observed in amended soil varied between 83 and 120% and was thus less pronounced than in autoclaved soil.

Discussion

Despite the economic importance of sugarcane industry in Egypt, some organic wastes such bagasse, press mud (filer mud/cake) and molasses are produced daily during the

manufacturing process and often accumulate causing a challenging problem regarding storage and adverse impacts of the environment. Therefore, most primitive factories in Upper Egypt commonly tend to sell press mud to contractors for use in agriculture as soil amendment or fertilizer. In most factories, bagasse is burnt for steam and power generation. The ash produced from the burning process is rich in nutrients and can be used efficiently as "bagasse ash" fertilizer in agriculture (Nakhla and El Haggag, 2014). The current work was therefore mainly focused on characterizing BA and PM as soil amendments by studying their effect on physico-chemical and biological soil properties. Another part of this study evaluated traits of the fungus *P. indica* potentially associated with disease control using the example of FW. Finally, experiments were performed in the greenhouse to study the effects of combined use of the soil amendments BA and PM and *P. indica*.

For proper decomposition in soil, it has been suggested that application of BA and PM to the soil should be one month prior to sowing (Dotaniya et al., 2016). Therefore, in the present study BA, PM and BA+PM, were added to the soil mixed well, and after irrigation with water to 75% soil moisture capacity the mixture was covered for 14 days. Afterward, the cover was removed and the mixture was left to dry for 2 weeks before starting the trials. It is clear from the results obtained that application of BA and PM alone or in a mixture improved the physical condition of the treated soil. One effect was the reduction in bulk density of the soil which ultimately increased the total porosity due to improvement of soil aeration, water retention and root development. This is similar with that was previously reported (Patil and Shingate, 1981; Kumar and Mishra, 1991; Rangaraj et al., 2007; Jamil et al., 2008; Sarwar et al.,

2010; Khan, 2011; Dotaniya et al., 2016). Also, application of PM at 2.5 and 5% slightly decreased the soil pH. The reduction in soil pH could be due to the effect of organic acids which produced during the decomposition of organic amendments (Jamil et al., 2008; Negim et al., 2016). This was in agreement with the reports by Clarson (1983), Kumar and Mishra (1991), Khan (2011) and Dotaniya et al. (2016). It is known that PM has a high content of nutrients and CaSO_4 , the latter acting as a vital soil amendment in alkaline soils (Tiwari et al., 1998). Although PM can reduce the soil pH, good results were obtained when it was applied in mixture with other pH reducing agents such as phosphorus fertilizer (Borde et al., 1984), gypsum (Reddy et al., 1973), gypsum + Zn (Chand et al., 1980) and pyrites (Singh et al., 1986). On the other hand, the increase in soil EC after addition of BA, PM and BA+PM, could be due to the high value of EC in organic amendments. The application of organic manures (FYM, Poultry manure and press mud) increased the electrical conductivity of the soil (Jamil et al., 2008; Ghulam et al., 2010; Khan, 2011; Negim et al., 2016). Soluble cation content was also affected (increased) by addition of BA, PM and BA+PM in all soil treatments compared with control. This may be due to the chemical composition of applied organic amendments (Jamil et al., 2008; Muhammad and Khattak, 2011; Negim et al., 2016). The application of organic amendments could be raising the amount of Ca, Mg, Na and K in soil solution due to the dissolved CO_2 and organic acid produced from organic amendments (Wong et al., 2009; Negim et al., 2016). Likewise, application of BA and PM to the soil up to 5% directly increased soil contents of organic matter, macro and micronutrients such as NPK, Fe, Zn, Cu and Mn. However, PM was more effective than BA and the mixture of the two resulted in the highest increase of nutrient content. Several studies carried out under pot or field conditions recommended application of BA and PM for improving soil chemical properties by increasing the organic matter and nutrients in the soil (Bokhtiar et al., 2000; Rangaraj et al., 2007; Jamil et al., 2008; Singh et al., 2008; Sarwar et al., 2010; Khan, 2011; Dotaniya et al., 2016). Availability of nutrients including an immediate P release were directly enhanced by the effects of the residues of these organic wastes (Datta and Gupta, 1983) or by indirect effects during decomposition of their residues in the soil which produced a range of organic acids (Dotaniya and Meena, 2013) causing mobilization of insoluble P (Dotaniya et al., 2013). The addition of BA and PM greatly increased bacterial and fungal population in the rhizosphere of cucumber plants when added to the soil alone or in a mixture. However, PM was more effective than BA, and the mixture resulted in the highest abundance of fungi and bacteria. Ownen (1954) also reported that application of PM to the soil greatly increased bacterial and fungal populations. Increases in soil microbial diversity and populations after addition of amendments are generally attributed to an increase in easily available carbon as a food material (Singh et al., 2009). It is also known that PM encourages growth of bacteria and microorganisms (Ownen, 1954; Ochao-George et al., 2010).

In order to evaluate the effect of BA and PM on Fo and *P. indica*, these fungi were cultured in liquid media amended with aqueous extracts of BA and PM. It was observed that the water extracts of PM and the extracts from BA and PM significantly inhibited the growth of Fo. In a previous study, the aqueous extract of 10% PM also inhibited *F. solani* linear growth (Pandya et al., 2009). Also, preparations from cashew (*Anacardium occidentale* L.) peduncle bagasse have shown an antifungal activity against *F. oxysporum*, *F. moniliforme* and *F. lateritium* (Santos et al., 2011). On other hand, the aqueous extracts of BA and PM significantly stimulated the growth of *P. indica* by increasing the mycelial mass production in our study. It has been earlier reported that PM is very rich in nutrients (Solaimalai et al., 2001; Gupta et al., 2011; Khan, 2011; Dotaniya et al., 2016) and encouraged the growth of various fungi including *Neurospora crassa*, *Trichoderma viride*, *Aspergillus* sp. and *Penicillium* sp. (Anonymous, 1968).

In our study we observed chlamydospores of *P. indica* within the root cortex cells of 14 and 28 day-old plants of cucumber after inoculation of seeds with *P. indica*. This is in agreement with the results of histological studies performed with the fungus on other plants colonized by *P. indica* such as wheat (Serfling et al., 2007; Yilmaz, 2013; Rabiey et al., 2015; Moharam et al., 2017), barley (Waller et al., 2007; Yilmaz, 2013), maize (Kumar et al., 2009; Rane et al., 2015) and rice (Bagheri et al., 2014). Colonization of cucumber roots with *P. indica* was also confirmed by PCR diagnosis. When PCR was performed with DNA extracted roots of inoculated plants, the PCR products were identical with the product (751-bp in length) obtained by PCR with DNA from a pure culture of *P. indica*. This was in agreement with earlier studies reporting detection of *P. indica* in colonized roots of wheat plants (Serfling et al., 2007; Moharam et al., 2017). To our knowledge, this is a first record of colonization of cucumber roots by this endophytic fungus. Moreover, in agreement with the positive effects of aqueous extracts of BA and PM on biomass of *P. indica* observed in vitro, the colonization rate of roots of cucumber with *P. indica* to was highly increased by up to 70 and 75% when the experiment was performed in soil amended with BA and PM. Taken together, this clearly showed a positive effect of PM and BA on *P. indica*.

As in other studies, a dual culture test was carried out for exploring the antagonistic activity of *P. indica* and Fo and vice versa *in vitro*. In this test, the inoculated plates were incubated at 28 ± 5 °C, the most favorable temperature for growth of both fungi. In these test, a clear antagonism between both fungi was not found. A lack of an antagonistic effect of *P. indica* on other species of *Fusarium* such as *F. culmorum*, *F. graminearum*, *F. verticillioides* or *F. oxysporum* causing root diseases on different plants has been reported before (Deshmukh and Kogel, 2007; Kumar et al., 2009; Rabiey et al., 2015; Moharam et al., 2017). Also, on agar media amended with culture filtrate of *P. indica* a negative effect on mycelial growth of Fo was not seen (data not shown), confirming the results of the dual culture test.

In the present pot experiments, a significant reduction in severity of FW was observed when seeds of cucumber

were treated with *P. indica* as compared to the disease severity of plants grown from non-treated seeds. The reduction was even more pronounced in soil amended with BA and PM alone with the mixture. Thus, the high reduction in disease severity observed in the pot experiment together with a clear lack of antagonistic activity of *P. indica* on *Fo* *in vitro* indicates an induction of disease resistance against FW by *P. indica*. A protection against root diseases caused by *Fusarium* spp. due to induction of resistance by *P. indica* has been reported before in different plants (Deshmukh and Kogel, 2007; Kumar et al., 2009; Rabiey et al., 2015; Moharam et al., 2017). Possibly, the disease reducing efficacy of the BA and PM amendments seen in the pot experiment was the result of a combined effect of the fungistatic properties of the amendments toward *Fo* on the one hand and an increase in the abundance of *P. indica*, especially in autoclaved soil amended with BA and PM, on the other. Another factor suspected to have contributed to the decrease in FW by the amendments was their positive effect on the microbial activity in the soil, resulting in increase populations of bacteria and fungi, particularly in the non-autoclaved soil amended with BA and PM. The increase of these microorganisms maybe has played a vital role in suppressing the pathogen in the soil. However, the role of soil biology for the mechanism of FW control after incorporation of BA and PM amendments along with *P. indica* in cucumber systems requires further investigation.

In this study, the biomass of vegetative growth (FrW and DrW g) of cucumber was improved after treating seeds with *P. indica* or application of BA and PM amendments to the soil alone or in mixture. However, the best vegetative growth was observed when seeds treated with *P. indica* were sown in autoclaved or non-autoclaved soil amended with BA and PM. Several previous studies have documented the role of this endophytic fungus as a growth promoter in a range of plants (Waller et al., 2005; Oelmüller et al., 2009; Zuccaro et al., 2009; Varma et al., 2012a; Varma et al., 2013; Bagheri et al., 2014; Moharam et al., 2017). Increases in plant growth and yield have been reported before (Borde et al., 1984; Singh et al., 1986; Jamil et al., 2008; Khan, 2011; Dotonyia et al., 2016).

Conclusions

From the present study it can be concluded that seed treatment with the endophytic fungus *P. indica* combined with the BA and PM soil amendments has the potential to improve soil properties. PM and BA showed positive effects on soil properties by increasing organic matter, increasing macro and micronutrients in the soil solution and by increasing the growth and resistance to FW of cucumber. Further experiments are needed to confirm that the identified treatments can manage the disease in the field.

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