



Piriformospora indica promotes cucumber tolerance against Root-knot nematode by modulating photosynthesis and innate responsive genes

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ABSTRACT

Root Knot Nematode (RKN, *Meloidogyne incognita*) is one of the greatest damaging soil pathogens causes severe yield losses in cucumber and many other economic crops. Here, we evaluated the potential antagonistic effect of the root mutualistic fungus *Piriformospora indica* against RKN and their impact on vegetative growth, yield, photosynthesis, endogenous salicylic acid (SA) and its responsive genes. Our results showed that *P. indica* dramatically decreased the damage on shoot and root architecture of cucumber plants, which consequently enhanced yield of infested plants. Likewise, *P. indica* colonization clearly improved the chlorophyll content and delimited the negative impact of RKN on photosynthesis. Moreover, *P. indica* colonization exhibited a significant reduction of different vital nematological parameters such as soil larva density, amount of eggs/eggmass, eggmasses, females and amount of galls at cucumber roots. Additionally, the results showed that SA level was significantly increased generally in the roots of all treatments especially in plants infested with RKN alone as compared to control. This suggests that *P. indica* promoting SA levels in host cucumber plant roots to antagonize the RKN and alleviate severity damages occurred in its roots. This higher levels of SA in cucumber roots was consistent with the higher expressional levels of SA pathway genes PR1 and PR3. Furthermore, *P. indica* colonization reduces PR1, PR3 and increased NPR1 in roots of RKN infested cucumber plants when compared to non-colonized plants. Interestingly, our *in vitro* results showed that direct application of *P. indica* suspension against the J2s exhibited a significant increase in mortality ratio. Our results collectively suggest that *P. indica* promoting morphological, physiological and SA levels that might together play a major important role to alleviate the adverse impact of RKN in cucumber.

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1. Introduction

Cucumber (*Cucumis sativus* L.) is vital economical vegetal crops (Mukhtar et al., 2013). In addition it consider a typical plant for vascular biology and sex determination studies with a draft genome size 243.5 Mb (~30% smaller than estimated genome size 367 Mb) (Huang et al., 2009). Root-knot nematodes (RKNs; *Meloidogyne* spp.) are endoparasites causing a dangerous hazard to agricultural sector. RKN includes more than 80 species, with a diverse devastating ability. Globally, root knot nematode is soil pathogens producing severe yield damages in numerous crops due to their ability to infest many of plant taxa (Abad and Williamson, 2010; Kayani et al. 2017). RKN species cause an estimated yearly damage of \$157 billion

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worldwide (Deau et al., 2008). Moreover, its induce typically root deformations (galls), leaf chlorosis, defoliation, dwarfing, wilting and dropping profit of infested plants (Sasser et al., 1983). Control of phytoparasitic nematodes is a major global challenge for growers all over the world. Various agricultural practises and biological approaches against RKNs have been testified (Collange et al., 2011). One of tactics for managing RKN in vegetable crops is crop rotation (Osei et al., 2010). But due to the wide host range of RKN, efficacy of crop rotation as a phytoparasitic nematode management strategy was decreased (Chen and Tsay, 2006; Salem et al., 2019a). Therefore, selection of resistant varieties would be a standard approach to defend plants against RKN (Guan et al., 2012). To date, no commercial cucumber cultivars are found to resist plant roots against *Meloidogyne* spp. Moreover, negative impact of synthetic pesticides and chemicals inputs on human and environment restrict their application globally (Nicolopoulou-Stamati et al., 2016). However, some approaches exhibited to cope plants against RKN parasitism such as cover crops (Navarrete et al., 2016), grafting (Liu et al., 2015; Salem et al., 2019b), application of organic amendments (Abdel-Dayem et al., 2012; Abdeldaym et al., 2014) and plant extracts (Javed et al., 2007) as well as bio-control (Daneshkhah et al., 2013; Atia et al., 2016). All these strategies either alone or combined together are considered the most sustainable methods for controlling plant root knot nematode (Escobar et al., 2010, 2015). However, controlling root knot nematodes is hard because its complicated life cycle and environmental threats of poisonous nematicides. During the last decade, a growing attention has paid to the application of plant growth-promoting fungi (PGPF) by way of a supportable and potential alternative to chemicals pesticides (Dutta and Thakur, 2017). Among antagonists fungi, the symbiotic mycorrhizal fungi has played an important role in the acquisition of nutrients from soils, stimulating plant growth, in addition to their ability to suppress the destructive effect of above and below-ground plant pathogens, resulting in an improvement of plant performance and their tolerance (Schmidt et al., 2011; Rai et al., 2014). *Piriformospora indica* is a plant-root-colonizing fungus belonging to division *Basidiomycetes*. It could be simply replicates in the absence of any host plant, in addition to its ability to full-grown at different compound and slight substrates, so its asexually stage forms chlamydospores (Varma et al. 1999). Its discovered in 1998 in the Indian Thar desert by Varma and his collaborators, *P. indica* has attracted great attention due to its growth and yield promoting ability, besides its potential to confer systemic resistance against many of biotic and abiotic stresses (Daneshkhah et al., 2013; Li et al., 2017; Xu et al., 2017; Abdelaziz et al., 2019). In addition to the direct beneficial interaction of *P. indica* with plant host, application of autoclaved culture filtrates (CF) containing fungal exudates as well as cell-wall extracts (CWE) were proved their powerful to promote plant growth (Vadassery et al., 2009). Furthermore, *P. indica* had resulted in an intense interest in the implementation of the fungus as biofertilizer and bioprotector (Venneman et al., 2017). Many studied stated the possibility of *P. indica* to improve plant resistance against powdery mildew (Qiang et al., 2012; Salem et al., 2018), *Fusarium oxysporum*, *Verticillium dahlia* and Pepino Mosaic Virus (Sarma et al., 2011; Fakhro et al., 2010; Sun et al., 2014). Therefore, the aim of the current study is to evaluate the potential antagonistic effect of *P. indica* against root knot nematode (*Meloidogyne incognita*) and their impact on greenhouse cucumber production. Additionally, to evaluate vegetative growth characteristics, photosynthesis analysis, salicylic hormone levels and some of innate responsive genes.

2. Materials and methods

2.1. Plant, nematode, fungus cultivation and inoculation

Cucumber Seeds (*Cucumis melo* cv. Hesham) superficial sterilized with 1.5% sodium hypochlorite up to ten min, washed 3 peri-

ods by H₂O, and then immersed again in SDW for 3 h. After that, placed in hot SDW at 60 °C for 5 min to kill any endophytes inside the seeds, then left for air drying (Huang and Backhouse, 2005). To test the absence of any internal endophytes, the sterilized seeds were sown on potato dextrose agar (PDA) medium. Fifty sterile seeds were spread in sterilized soil mixture of peat moss: sand: vermiculite (1:1:1, v:v:v) at quadrangular elastic pots (7x7x8 cm) till germinate to be used as host for *M. incognita*. To prepare inoculum of *P. indica*, the fungus was cultured on PDA plates at 28 ± 5 °C and then incubated in PDA broth medium for half month in 29 °C speed 155 rpm on a shaker. Mycelium of grown fungus was collected, centrifuged and washed 3 times with SDW. Twenty grams of *P. indica* mycelium was mixed with 1000 ml SDW to produce 2% *P. indica* suspension. To prepare inoculum of nematode, *Meloidogyne Incognita* eggs in this test were extracted from infested pumpkin roots (*Cucurbita pepo* L.) according to Hussey and Barker (1973). The second-stage juveniles (J2s) from eggs were collected and stored at 15 °C till used. The number of J2s in 1 ml SDW was adjusted to 100 J2s/ml.

2.2. Greenhouse experiment

Pot experiment was carried out under greenhouse, controlled conditions at 28 ± 2°C for two successively seasons (2016/2017) at Faculty of Agriculture, Cairo University. The treatments were as follows: Control (CT; without nematode infestation and without *P. indica* inoculation), *P. indica* (PI; with *P. indica* inoculation only), Nematode (RKN; with nematode infestation only) and *P. indica* with nematode (RKN + PI; with *P. indica* inoculation and nematode infestation). For *P. indica* inoculation, 10 ml of 2% *P. indica* suspension was injected in the root zone for each plant at 3, 7 and 15 days in nursery before transplanting, whereas SD used as a fake test. Afterward, the cucumber seedlings were transplanted in formalin-sterilized polyethylene pots (20 cm) field with sandy soil and each pot was contained one plant. All treatments were distributed randomly and all trials were replicated 40 times. Afterward 14 days of transplantation, pots of both treatments (RKN and RKN + PI) were inoculated with the J2 at the rate of 1000 J2/pot. Plants were irrigated twice weekly and were fertilized once intervals with ½ Hoagland's nutrition solution to sustain their growth. For nematological analysis, by the end of experiment, cucumber roots were uprooted, washed and number of galls per plant was assessed according to scale of Barker (1985). The number of egg masses isolated from cucumber roots was accounted under microscope (Hussey and Barker, 1973). The J2 population at the soil after each pot was determined by sampling 50 g of soil by means of Cobb's sieving and transferring protocol, after the adapted Baermann funnel protocol (Southey, 1986). Then, total number of J2 per kilogram of soil was calculated.

2.3. Laboratory experiment

In vitro test was conducted twice to investigate the antagonistic activity of *P. indica* on mortality of *M. incognita*. Dual culture experiment was performed in Petri dishes inoculated with 10 ml of 2% *P. indica* solution plus 1000 J2s/ dish. Dishes inoculated with *M. incognita* or *P. indica* alone were served as controls. Then, all dishes were incubated at 28 ± 5 °C for 7 days and afterwards survived J2s were counted at 0, 3, 5 and 7 days. Additionally, parallel experiment was conducted twice to evaluate the influence of defense-phytohormone (SA) against larvae of *Meloidogyne incognita*. Dishes containing 1000 J2s/dish were inoculated with SA alone (500 μM of SA) or combined with 2% *P. indica* suspension. The number of mortality of J2s was counted at 1, 2, 3 and 7 days.

2.4. Vegetative growth and total yield assessment

Twenty-four plants of each treatment were selected randomly to determine cucumber plant growth and development parameters. Plant height, fresh and dry masses of treated cucumber plants were calculated after 30 days of nematode infestation. Leaf area meter (C1-202 Laser Areameter) was used to measure area of the 5th leaf from top. Fruits of each treatment were daily harvested and weighted till the end of the experiments to calculate the average total yield per meter-square.

2.5. Physiological analysis

2.5.1. Chlorophyll content, photosynthesis, transpiration rate and water use efficiency

After 35 days of RKN infestation, the 5th leaf of 24 plants from each treatment was selected for physiological analysis. Chlorophyll content of was calculated by Chlorophyll meter (SPAD, 502 Minolta Co, Japan). Photosynthesis rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and H_2O usage efficacy (WUE; $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$) were measured at mid-day (12:00 am – 14:00 pm) using an infrared gas analyzer LICOR 6400 (Lincoln, Nebraska, USA).

2.6. Endogenous level of salicylic phytohormone

Root of cucumber plants were gathered in liquid nitrogen and kept in freezer. Samples were then ground and 10 mg of powdered tissue was isolated with 9:1 (v/v) methanol-chloroform, then derivatized by BSTFA at 120 °C for 1 h, Forcat et al. (2008). Quantitative study was done by using GC-MS instrument in SIM mode using an inner standard for SA.

2.7. RNA isolation and quantitative PCR analysis

Thirty-five days post RKN infestation, leaves and roots were collected from three replicates in liquid nitrogen for each treatment. RNA was isolated with Trizol reagent and treated with DNase I (Cat Num.: EN0525, Thermo Scientific). The cDNA was synthesized using the SuperScript™ II Reverse Transcriptase as outline by manufacturer's manual (Cat Num.: 18064014, Thermo Scientific). Gene-specific primers (NPR1, PR1 and PR3: for SA pathway; and Actin as a housekeeping gene were used (Table 1S). The qPCR examination was done using an Mx3000P QPCR System (Agilent Technologies). 15 μL final volume of the reaction. As outlined by (Beaubois et al., 2007). Three biological replicates (3 plants/treatment) were evaluated, and the mean and standard deviation values of statistics were measured.

2.8. Statistical analysis

All experiments and treatments were carried out in three replicates, each replicate consist of 24 plants for greenhouse experiment and 24 dishes for *in vitro* trial. Data of the two successive seasons (2016 and 2017) were exposed to analysis of variance (ANOVA) and means were linked by Duncan examination ($p < 0.05$) using the statistic 7 program (version 2004). The significance of differences among gene expressions data sets were assessed using paired student's *t*-test based on three replicates.

3. Results

3.1. Effect of *P. Indica* colonization on vegetative performance

Generally, *P. indica* has a significant positive influence on shoot and root architecture of cucumber plants; which consequently enhance the vegetative growth through motivation to produce more roots and branches (Fig. 2a, b). Particularly, the RKN + PI plants (simultaneously inoculated with *M. incognita* and *P. indica*) were less affected by nematode infection and exhibit significant higher levels of leaf area, plant high, fresh and dry biomasses comparing to plants inoculated with *M. incognita* alone (RKN plants) (Fig. 1a,b). Obviously, we showed an important rise, *i.e.* 1.5-fold in plant height; 1.7-fold in both fresh weight and leaf area; and 1.9-fold in dry weight in cucumber plants simultaneously colonized with *M. incognita* and *P. indica* related to the plants injected with *M. incognita* alone (Fig. 2c, d, e). Furthermore, we observed non-significant increase in plant height (1.2-fold); fresh weight (1.0-fold) in cucumber plants colonized with *P. indica* alone (PI) related to the control plants (CT), (Fig. 2 c,e,f). Meanwhile, we found significant increase in both dry weight (1.4-fold), leaf area (1.2-fold) in cucumber plants colonized with *P. indica* alone related to the control.

3.2. Effect of *P. indica* colonization on yield

The conducted experiment results revealed that RKN + PI plants produced relatively higher yield comparing to RKN plants suggesting that even the PI enhancing the plant resistance against the RKN infestation or that PI directly affecting the Root-knot nematodes viability and productivity; and both two suggestions will be examined in our study (Fig. 3). The fruits yield was found to increase by 38% (in PI vs CT treatment) and increase by 167% (in RKN + PI vs RKN treatment). This increase in total yield found to be positively correlated with the increase in all tested vegetative growth parameters (leaf area, plant high, root length, fresh and dry).

3.3. Effect of *P. indica* colonization on chlorophyll content and photosynthetic parameters

The effect of *P. indica* inoculation on chlorophyll content (SPAD), photosynthesis rate (P_n) and water use efficiency (WUE) as given in Fig. (4a, b, d) clearly indicated that, plants inoculated with *P. indica* alone (PI) significantly showed higher records comparing to control (CT). Meanwhile, plants infested with RKN alone (RKN) showed significant reduction in these parameters as compared to all other treatments. Fascinatingly the interaction effect on the simultaneously inoculated plants with both RKN and PI (RKN + PI) resulted in significant rising of chlorophyll content, photosynthesis rate and water use efficiency over plants infected with RKN alone (RKN).

On the other hand, there was no significant change among the inoculated plants with PI alone (PI) and un-inoculated (CT) with respect to the transpiration rate (C_i). While the plants infested with RKN alone (RKN) showed significant rising in their transpiration rate parameters as compared to other treatments. Interestingly, the plants simultaneously inoculated with both RKN and PI (RKN + PI) revealed a clear reduction in their transpiration rate compared to plants infested with RKN alone (RKN) (Fig. 4c).

3.4. Effect of *P. Indica* colonization on defense-phytohormone (SA)

Plant-microbe interactions are commonly correlated with alterations in levels of phytohormones. In order to investigate whether

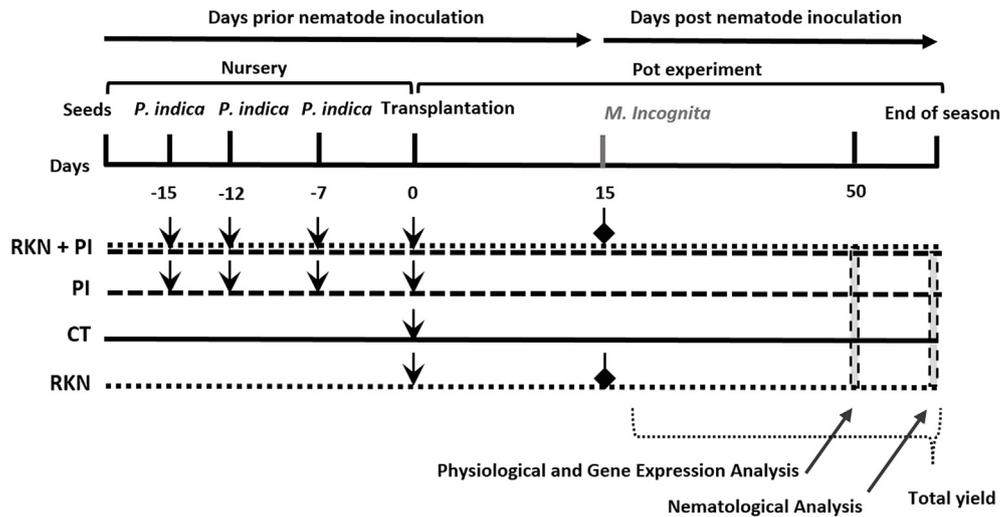


Fig. 1. Scheme of the experimental set up. Horizontal lines characterise different plant treatments as follow: Control (CT), *P. indica* inoculation (PI), nematode infestation (RKN), and dual inoculation of *P. indica* and *M. incognita* (RKN + PI). Samples for physiological and gene expression analysis were collected at 35 days after inoculation with *M. incognita*, while nematological analysis were done at the end of experiments. Cucumber fruits were harvested daily and were weighted till the end of experiments to calculate the total yield per plant.

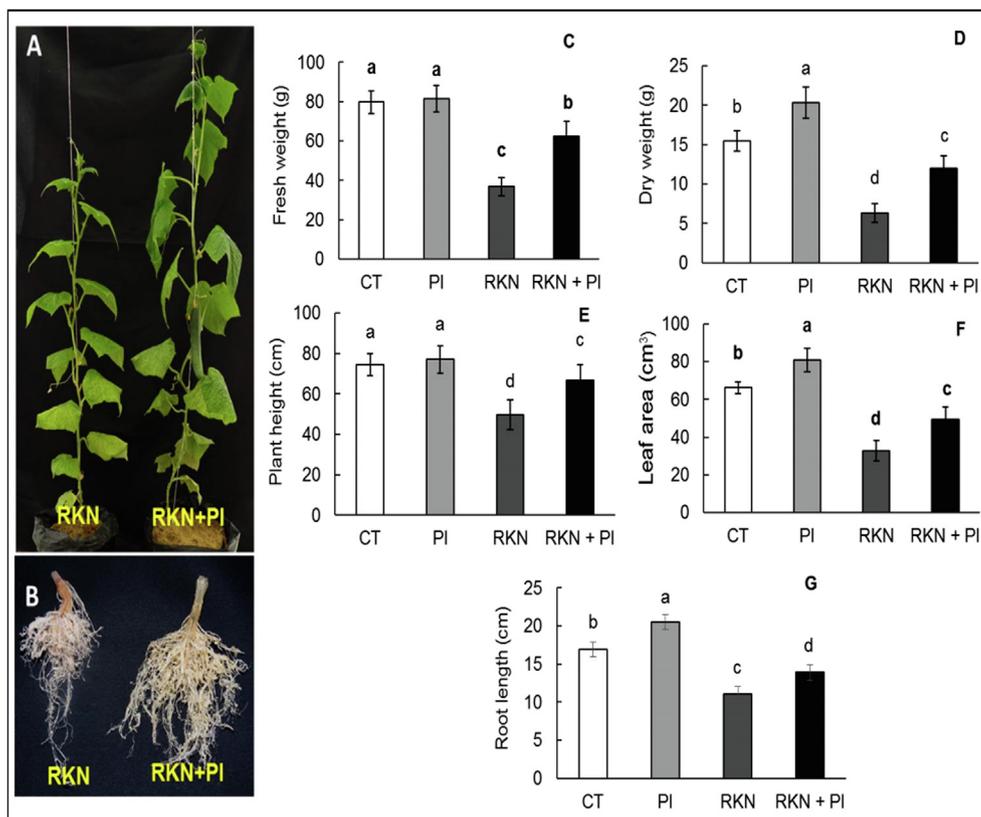


Fig. 2. Effect of *P. indica* colonization and/or RKN infestation on cucumber architecture. Treatments were arranged as follow: control (CT), *P. indica* inoculation (PI), nematode infestation (RKN), and dual inoculation of *P. indica* and *M. incognita* (RKN + PI). (A) and (B) photos represent the difference in the vegetative growth and root architecture of cucumber plants under RKN infestation alone or in combination with *P. indica* colonization, respectively. Fresh weight (C), dry weight (D), plant height (E) leaf area of the 5th leaf (E), root length (G) were measured at 35 post RKN infestation. Columns with the similar letter are not significantly dissimilar rendering to Tukey test ($p < 0.05$).

the *P. indica* affects the level of defense-phytohormones, the amounts of SA was measured across all treatments including control. Interestingly, our results demonstrated that the defence-phytohormone SA is significantly up-regulated in plant roots inoculated with *P. indica* alone (PI) comparing to control (CT) plants. As a response of cucumber plants infested with RKN alone,

we showed a significant rise, i.e. 24-fold in SA hormone level as compared to control. On the other side, as a result of colonization with PI of the cucumber plants infested with RKN, we clearly observed that PI colonization successfully decreased level of SA hormone (–7)–fold in roots as compared to plants infested only with RKN (Fig. 5).

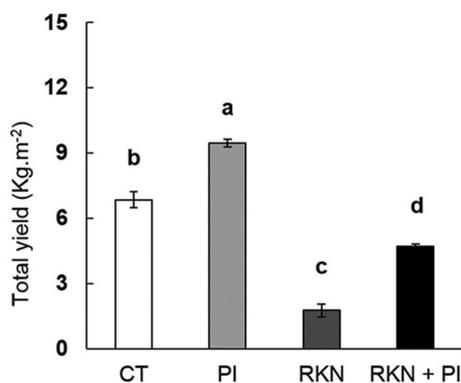


Fig. 3. Effect of *P. indica* colonization and/or RKN infestation on cucumber fruit yield. Treatments were arranged as follow: control (CT), *P. indica* inoculation (PI), nematode infestation (RKN), and dual inoculation of *P. indica* and *M. incognita* (RKN + PI). Columns with the similar letter are not significantly dissimilar rendering to Tukey test ($p < 0.05$).

3.5. Effect of *P. indica* colonization and SA on *M. incognita* eggs and J2

In vitro experiments, the obtained results showed that the direct application of *P. indica*, and SA separately or combined together significantly increased the mortality ratio of J2. After 7 days of exposure period, the highest mortality ratio in J2 population density was achieved by mixing *P. indica* suspension with salicylic acid (75.8%), followed by *P. indica* alone (69.3%), and then by salicylic acid alone (48.3%), as compared with the control (1.6%) at 7 days (Fig. 6). On the other hand, the obtained results from *in vivo* experiment indicated that soil larva density, developmental stages, no. of eggs/eggmass, no. of eggmasses, no. of females in root and no. of galls were significantly decreased in RKN + PI vs. RKN treatment (Fig. 7).

3.6. Effect of *P. indica* colonization on expression levels of SA related genes

The effect of *P. indica* inoculation on expression levels of NPR1, PR1, PR3 genes in roots clearly indicated that PI showed significant

higher records (*i.e.* 7-fold for NPR1 and 0.7-fold for PR1) comparing to control (CT). While, a slight reduction in the expression level was observed in PR3 gene. Meanwhile, plants infested with RKN alone (RKN) showed a important significant rise in the expression levels of both PR1 and PR3 as compared to other treatments. On the contrary, the NPR1 expression level was significantly down-regulated as compared to all other treatments except the CT. Interestingly, the interaction effect on the simultaneously inoculated plants with both RKN and PI (RKN + PI) resulted in significant rising of NPR1 expression over plants infected with RKN alone (*i.e.* 2.5-fold). Conversely, the effect of simultaneous inoculation with both RKN and PI resulted in significant mitigation of PR1 and PR3 expression levels when compared to plants infected with RKN alone (*i.e.* -1-fold and -0.2-fold, respectively) (Fig. 8).

4. Discussion

Global concerns about the overusing of synthetic pesticides have given a great attention to looking for new environmentally-friendly nematicides. The utilization of useful antagonistic microorganisms as mycorrhizal fungi, rhizobacteria and endophytic bacteria against phytoparasitic nematodes has been considered as a promising alternative to the globally available toxic nematicides (Le et al., 2009; Vos et al., 2012). In this regard, a powerful biocontrol fungus; nonpathogenic *P. indica* was described to positively promote and improve the response of different biotic and abiotic stresses in many crops. Therefore, this study was conducted to investigate the antagonistic power of *P. indica* against *Meloidogyne incognita* and their powerful impact on cucumber agro-physiological parameters as well as some molecular innate responsive genes. Overall results clearly showed a suppressive effect of *P. indica* on RKN infestation as well as enhancing the host plant agro-physiological characteristics through modulation of different host responsive genes. Regarding the vegetative growth characteristics, in a completely agree with previous reports, our results confirmed that the beneficial root endophyte *P. indica* significantly enhancing plant height (*i.e.* 1.2-fold), leaf area (*i.e.* 1.6-fold), root length (*i.e.* 1.3-fold), fresh and dry weight (*i.e.* 1.9-fold and 2.6-fold, respectively) in dual infested plants (RKN + PI) as compared to RKN

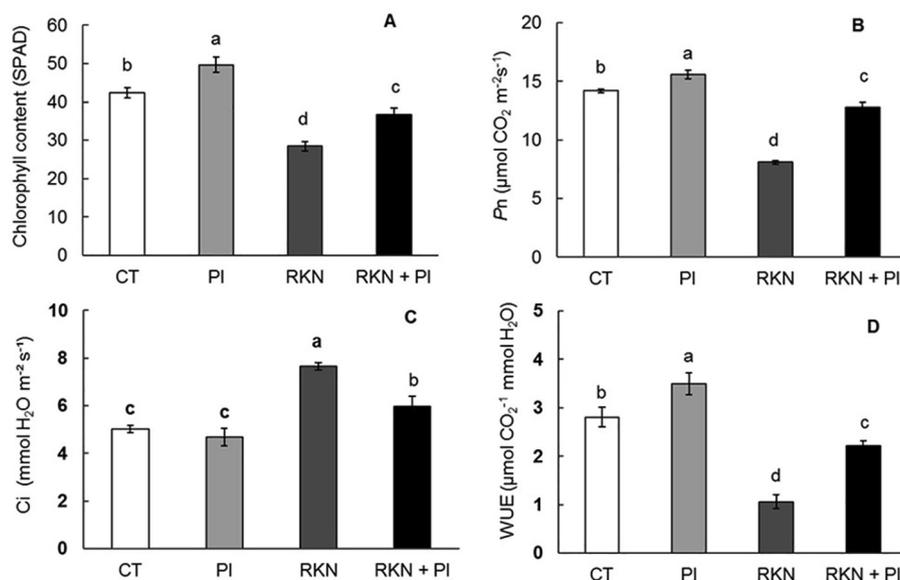


Fig. 4. Effect of *P. indica* colonization and/or RKN infestation on (A) estimated chlorophyll content (SPAD), (B) photosynthesis rate (P_n), (C) transpiration rate (C_i) and (D) water use effectiveness (WUE) at 35 days post RKN infestation. Treatments were arranged as follow: control (CT), *P. indica* inoculation (PI), nematode infestation (RKN), and dual inoculation of *P. indica* and *M. incognita* (RKN + PI). Columns with the similar letter are not pointedly dissimilar according to Tukey test ($p < 0.05$).

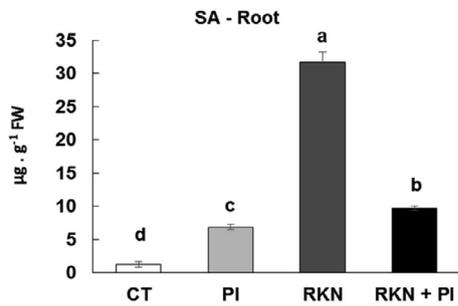


Fig. 5. Effect of *P. indica* colonization and/or RKN infestation on salicylic acid content (SA) in roots of cucumber; control (CT), *P. indica* inoculation (PI), nematode infestation (RKN), and dual inoculation of *P. indica* and *M. incognita* (RKN + PI). Columns with the similar letter are not significantly different according to Tukey test ($p < 0.05$).

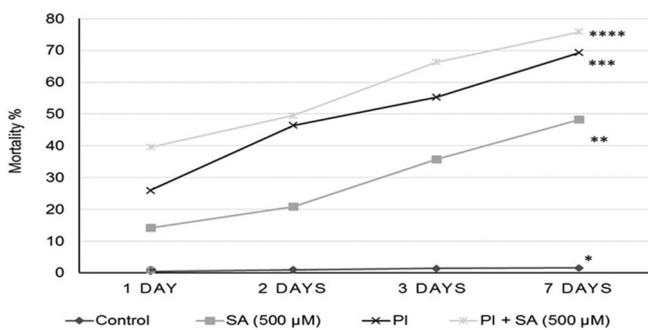


Fig. 6. An *in vitro* experiment shows the effect of direct applications of *P. indica* suspension (PI) and salicylic acid (SA) on mortality percentage of J2. * No. of stars indicate the degree of significance as related to control at $p < 0.001$ (****), $p < 0.01$ (**) and $p < 0.05$ (*) according to Tukey test.

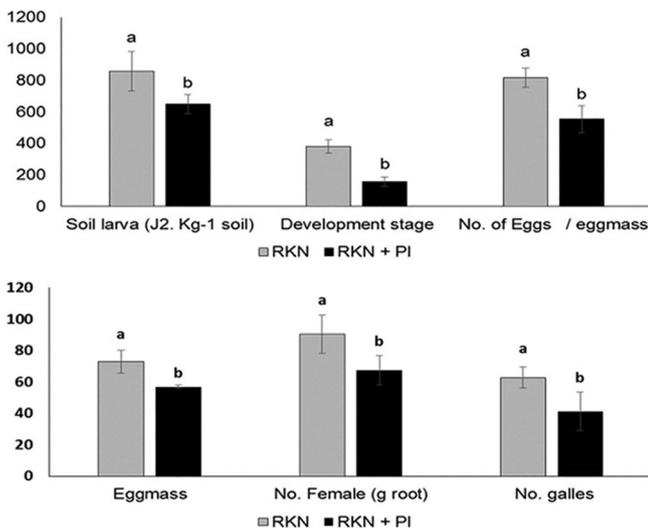


Fig. 7. Effect of *P. indica* colonization on soil larva density, developmental stage, no. of eggs/egg mass, no. of egg masses, no. of females and no. of galls. RKN = plants infested by nematode alone; RKN + PI = plants inoculated by both *P. indica* and RKN. Columns followed by the same letter are not pointedly dissimilar as to Tukey test ($p < 0.05$).

plants. This growth promotion not only might be attributed to enhancement in water absorption, nutrients uptakes, and physiological processes but also to the modulation of phytohormones as well as alleviation of RKN infestation (Fig. 2 and Fig. 4). The similar findings were observed by (Achatz et al., 2010; Ansari et al., 2013;

Bajaj et al., 2015; Xu et al., 2018). Furthermore, infested plants with RKN alone appeared in lowest fruit yield and plant growth (Fig. 3). This possibly is due to high population density of RKN attack root of cucumber plants and impaired of nutrient uptake and water absorption (Melakeberhan et al., 1987; Bartlem et al., 2014; Strajnar et al., 2012). Contrary, it has been found that *P. indica* colonization enhanced cucumber yield by increasing both number and weight of fruits generally as similar findings by Fakhro et al. (2010). On the other hand, RKN infestation clearly reduced chlorophyllous pigment (total; SPAD) and impaired the different photosynthetic parameters (photosynthesis rate, and water use efficiency). Conversely, the *P. indica* colonization improved the chlorophyll content (in case of control) and delimited the negative impact of RKN on photosynthetic parameters (in case of RKN + PI). Alike remarks have been documented previously on injection with *P. indica* (Arora et al., 2016; 2018). Interestingly, the *P. indica* also alleviated the sever effect of RKN on transpiration rate and chlorophyll contented might caused in rise biomass accumulation in the tested black pepper plants (Anith et al., 2018). In this respect also, it had reported that phytoparasitic nematodes induced creation of reactive oxygen species (ROS) that could be decompose the photosynthetic pigment (*i.e.* chlorophyll) as well as impairment of photosynthetic equipment, decrease of electron transport, carbon fixation capacity and photophosphorylation (Gillet et al., 2017). Interestingly, the reduction in value of photosynthesis rate of RKN plants was also strongly correlated leaf chlorophyll concentration, which consequently limits the gas exchange as many previous reports described (Loveys and Bird, 1973; Bowden and Rouse, 1991; Abouseadaa et al., 2015). On the other side, a significant reduction in nematological parameters includes, soil larva density, developmental stage, no. of eggs/egg-mass, no. of egg masses, no. of females in root and no. of galls in duel colonized plants (RKN + PI) as compared to plants colonized with RKN alone. The mechanism of control RKN using *P. indica* is still unclear enough. Few studies reported that phytoparasitic nematode reduction induced by *P. indica* colonization could be attributed to physical barriers formed by its hyphae reduced J2 mobility or syncytium expansion. Likewise, it might be also attributed to the release of fungus nematicidal compounds mixture that significantly reduced egg hatching and J2 mortality (Daneshkhah et al., 2013). Numerous reports have elucidated that fungal endophytes might change chemical possessions of root exudates or may inspire plants to yield chemicals or hormones which repel or disturb nematode attraction (Le et al., 2009). Likewise, repellent impact of root exudates of tomato plants tested with beneficial endophytic *Fusarium oxysporum* against *M. incognita* was described (Dababat and Sikora, 2007). Interestingly, the *in vitro* experiment showed that the direct application of salicylic acid against the J2s of *M. incognita* exhibited a significant increase in mortality ratio, which might be explain 70% of the *P. indica* effect to antagonize the root knot nematode. However, biotic stress defense hormones are considered a part of complex network of synergistic and antagonistic interactions (Fraire-Velázquez et al., 2011). Salicylic is a key player in the stimulation of dissimilar signing paths in resistance feed back in plants, when they encounter nematode infections (Ali et al., 2018). It improves the host immune response, compromises the plant defense system whereas and reported to enhance a set of pathogenesis-related defense genes against the phytoparasitic nematode in tomato (Branch et al., 2004) and the suppression of host resistance SA was connected with the development of nematode in tomato (De Medeiros et al., 2017). Under greenhouse conditions, our obtained results showed that the trend of SA significantly increased in the roots of all treatments as compared to control. These results suggest that *P. indica* promoting SA production inside the host cucumber plant post inoculation. However, the maximum level of SA was observed in plants infested with RKN

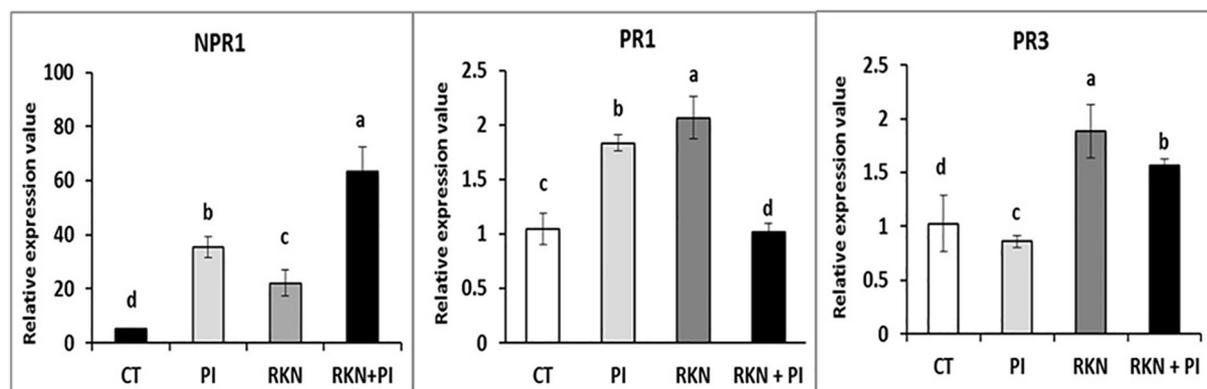


Fig. 8. Relative gene expression of NPR1, PR1 and PR3 genes in roots of the *P. indica* colonized and non-colonized cucumber plants at 35 days post RKN infestation. Significance of differences, $P < 0.001$ (**), between means was evaluated by Student's *t*-test, \pm SE ($n = 4$), based on three independent experiments.

alone, due to sever damages occurred in its roots. These high levels of damage in roots may stimulate plants to produce more SA as a defense mechanism to reduce population of nematode (Molinari et al., 2014). On contrast, a significant reduction in SA level was observed in dual inoculated plants (RKN + PI) as compared to plants infested with RKN alone. This reduction might indicate the significant effect of *P. indica* to mitigate the *M. incognita* population density and consequently injures severity due to RKN + PI interaction. Moreover, other studies confirmed that *P. indica* stimulated the production of salicylic acid that greatly decreased the phytoparasitic nematodes (*Heterodera schachtii*) development in plant roots (Daneshkhah et al., 2013).

Indeed, SA is a controller of plant resistance to biotrophic and hemibiotrophic pathogens. Molinari and Baser (2010), also confirmed that SA application delayed root knot nematode development and impaired egg masses production. Similarly, inducing systemic resistance against *M. incognita* (Kofoid & White) Chitwood, by SA addition caused a significant reduction in the reproductive parameters of the root nematodes in root (Mostafanezhad et al., 2014). These results agree with other previous investigations (Molinaria and Offredo, 2006; Mukherjee et al., 2012; Pu et al. 2014; Szitenberg et al. 2017; Tomalova et al. 2012).

In fact, one of the most distinctive defense gene dependent SA-signaling involved in plant immunity pathway is NPR1. So, this gene is necessary for the activation of pathogenesis-related PR genes such as PR1-a in cucumber during infection plants against nematodes in term of leading to hypersensitive response (HR) and systemic acquired resistance (SAR) in plants against biotrophic pathogens (Sahebani et al., 2011). Our result revealed that *P. indica* stimulated expression of defense genes related to salicylic acid in cucumber plants, in agreement with an earlier reports in which a genes of SA was strongly elevated in roots of nematode-attacked (Wu et al., 2012; Molinari et al., 2014; Hajipour et al., 2015). However, the level of SA reduced in (RKN + PI) plants, but the level was not reached to neither control (CT) nor plant treated with *P. indica* alone (PI). Many studies clearly reported that SA is an essential regulator of plant immunity as a plan to persuade the good resistance responses (Vlot et al., 2009; Thaler et al., 2012). Our study indicated that higher levels of expression of SA genes putatively linked to improvement of stress responses as induced by both *M. incognita* and *P. indica* in cucumber plants.

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Author Contributions

MEA, MAMA, EAA, GO and MTA designed the experimental. MEA and EAA carried out the greenhouse experiments and agro-nomical analysis. DSI, IS and EAA performed the nematological analysis. MA and IA performed the physiological analysis. MAMA and MTA conduct the genes expression work. MAMA and EAA wrote the manuscript. MEA coordinated the project.

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Declaration of Competing Interest

None declared.

Appendix A. Supplementary material

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