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Piriformospora indica promotes plant growth, reduces lodging and enhances seed yield, and quality in onion (*Allium cepa*)

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The plant rhizosphere is a complex realm where diverse microorganisms engage in various interactions with their host plants. These interactions can be mutually beneficial or parasitic, influencing various plant processes (Bhattacharyya and Jha 2012). Bioagents like Pseudomonas, Bacillus, Trichoderma, and vesicular arbuscular mycorrhiza (VAM) are used to improve crop growth. *Piriformospora indica*, a basidiomycete root endophyte, has a wide host range. Unlike VAM, *P. indica* can be cultivated in the lab and has shown potential in mitigating salinity stress, drought resistance, and enhancing nutrient uptake (Singh *et al.* 2003). Several studies report the beneficial role of VAM and Trichoderma which include disease management for purple blotch, white rots, and fusarium basal rot (Praveenkumar and Hegde 2017). There are several reports on the use of bioagents agents like VAM and Trichoderma in onion (*Allium cepa* L.). These studies report that *P. indica* treatment has benefits, such as enhancing onion seed germination and resistance to diseases like stemphylium blight (Singh *et al.* 2003 and Varma *et al.* 2012).

The crop stand and seed quality are affected by several fungal and bacterial rots caused by fusarium basal rot (*Fusarium oxysporum* f. sp. cepae), pink root disease (*Setophoma terrestris*) and white rot (*Stromatinia cepivora* (Berk.) Whetzel, 1945) and bacterial rot (*Erwinia carotovora* subsp. carotovora) (Yalamalle *et al.* 2019b, Chauhan *et al.* 2023). The conventional method of disease control involves the application of a large quantity of pesticides to the soil/plant, which has resulted in environmental pollution and induced pesticide resistance (Yalamalle *et al.* 2019a, Deosi and Suri 2022). Despite chemical treatments management of lodging is difficult (Yalamalle *et al.* 2020a). Since the onion is pollinated by honey bees, the use of chemicals in onion seed crop must be kept at a minimum. Considering the beneficial role of *P. indica* reported in other crops we studied the effect of *P. indica* treatment in onion seed crops. The present study reports for the first time the beneficial role played by *P. indica* in onion seed crop.

The field experiment was conducted during 2020–21 at ICAR-Directorate of Onion and Garlic Research, Pune, Maharashtra. Experiment was conducted in a randomized complete block design (RCBD) with 3 replicates. Each treatment block, measuring 10 m × 5 m, was planted with 60 onion bulbs (60±5 g each) soaked in a 50 litre solution with a biocontrol agent for 15 min and dried overnight. The *T. viride* was procured from T. Stanes and Company Ltd. India, while *P. indica* was obtained from National Institute for Plant Genome Research, New Delhi. The chlamydospore concentration for bulb treatment was adjusted to 1 × 10^5/mL. The dry formulation of VAM was procured from Zydex Industries Pvt. Ltd, Gujarat, India, and applied at a rate of 3 g/L. Carbendazim 50% WP (Crystal Crop Protection Limited, Delhi, India) was used at a rate of 2 g/L. Streptocycline® (streptomycin sulphate: 90% w/w + Tetracycline hydrochloride: 10% w/w, Hindustan Antibiotics Limited, Pune, India) was used at a rate of 0.5 g/L. Onion root colonization by *P. indica* was conducted as previously described (Roylawar *et al.* 2021).

Plant height was measured at 60 days after planting (DAP). Scape length and diameter were recorded at 50% anthesis. The filed observation was taken as average of 10 randomly selected plants. The seed germination and seed vigour were determined as described previously (Yalamalle *et al.* 2020b). ANOVA tables were formed by Fishers' least significant difference method at confidence ($P<0.05$). Per cent data was arcsine transformed prior to analysis.

Onion seed crop is susceptible to various fungal and bacterial diseases, resulting in lodging, reduced yield, and poor seed quality (Yalamalle *et al.* 2020a). Chemical pesticides, while commonly used, raise concerns about human health and the environment (Yalamalle *et al.* 2019a). In contrast, the environmentally friendly biological control

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approach utilizing bioagents, such as *P. indica*, VAM, and Trichoderma was employed in this study to evaluate its effects on plant stand, seed yield, and seed quality parameters in onion seed crop.

Effect of bioagents on plant stand: At 30 DAP, the highest plant stand (99.46%) was observed in the carbendazim treatment, statistically similar to tetracycline, *T. viride* and *P. indica* treatments (Table 1). Bioagents, living entities, require time to establish root colonization, with their maximum effectiveness achieved when roots are fully colonized. *T. viride* doesn't need root colonization, unlike VAM and *P. indica*, which do colonize the roots (Contreras-Cornejo *et al.* 2014). In contrast, chemicals like carbendazim and streptocycline act immediately.

At 60 DAP, carbendazim treatment had a plant stand of 88.17%, followed by *P. indica* (87.10%) (Table 1). Pre-sowing chemicals have both protective as well as curative functions. For instance, metalaxyl seed treatment reduced the incidence of white rot garlic (Alem Gebretsadik and Girmay Gebresamuel 2015). Seedling treatments with *T. viride*, VAM and *P. indica* have all showed improvements in germination and establishment in onion (Coşkuntuna and Özer 2008, Tsimilli-Michael and Strasser 2013).

Table 1 Effect of onion bulb treatment with different bioagents on plant stand and umbel diameter

Treatment	Establishment 30 DAP (%)	Establishment 60 DAP (%)	Umbel diameter (cm)
T. viride	97.85 (83.10) $^{ab\wedge\$}$	82.26 (65.54) ^{a}	4.92 ^{ab}
VAM	95.70 (78.20) ^{bc}	83.33 (66.07) ^{a}	4.76 ^{bcd}
P. indica	98.39 (84.12) ^{ab}	87.10 (68.98) ^{a}	5.01$^{a\$}$
Carbendazim	99.46 (87.57) ^{a}	88.17 (70.52) ^{a}	4.81 ^{abc}
Tetracycline	98.39 (84.12) ^{ab}	87.10 (69.02) ^{a}	4.57 ^{cd}
Control	89.78 (71.44) ^{c}	68.82 (56.08) ^{b}	4.53 d
CD (*P*=0.05)	8.02*	8.51*	0.24**

DAP, Days after planting.
$^{\wedge}$Values in the parenthesis are arcsine transformed values. $^{\$}$Different superscript letters indicate significant differences (*P*<0.05). CD, Critical difference. Significance code: *, *P*<0.05. DAP, Days after planting.

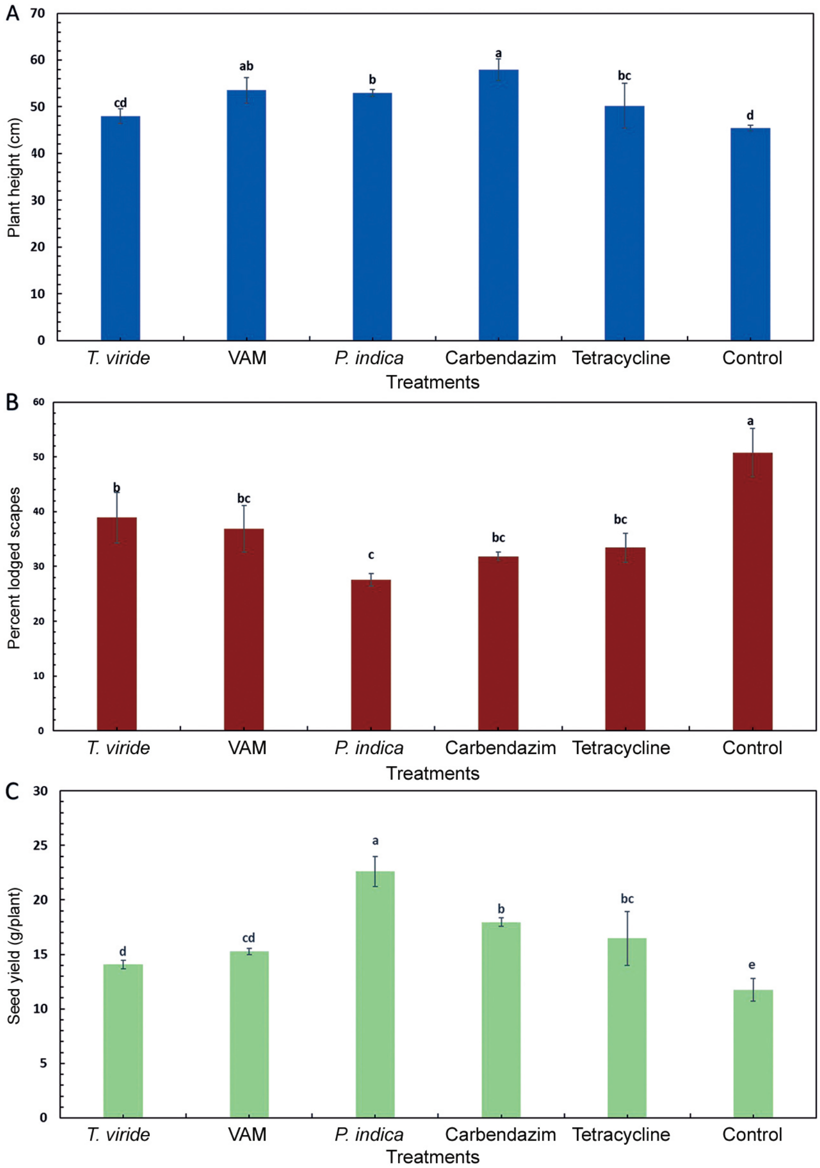

Fig. 1 Effect of onion bulb treatment with different bioagents on (A) Plant height; (B) Per cent lodged scapes; (C) Seed yield.
Different superscript letters indicate significant differences (*P*<0.05). Error bars represent standard deviation (n=3).

Effect of bioagents on vegetative traits and lodging: Onion bulb treatment significantly enhanced the plant height (Fig. 1A). The enhancement ranged from 2.53 cm to 18.13 cm, with the highest plant height was observed in carbendazim-treated bulbs, followed by VAM and *P. indica* treatments. Treatment with bioagent has been reported previously to enhance the production of growth promoters like NAA, GA (Singh *et al.* 2003), which might have contributed to enhancements. The tiller production is a genetic factor (Yalamalle V R 2016) therefore, the treatments may not have had a significant impact on the number of tillers/plant. Lodging presents a significant challenge for onion seed growers, often resulting from bulb and root rot caused by pathogens such as *Fusarium oxysporum* f. sp. cepae, *Setophoma terrestris, Stromatinia cepivora* (Berk.) Whetzel, 1945 and *Erwinia carotovora* subsp. carotovora (Schwartz and Mohan 2016, Yalamalle *et al.* 2019b). All treatments led to a significant reduction in lodging incidence, with the lowest lodging observed in plants treated with *P. indica,* VAM, tetracycline and carbendazim (Fig. 1B). *P. indica* is biotroph that resides within the host's roots until crop maturity. It has been reported to provide mechanical strength, harvest index

Table 2 Effect of onion bulb treatment with different bioagents on seed quality

Treatment	1000-seed weight (g)	Germination (%)	Seedling length (cm)	Seedling dry weight (mg/seedling)	Vigour index-I	Vigour index-II
T. viride	3.50 [b$]	73.33 (59.00)[bc^]	9.45 [ab]	2.18 [b]	692.97 [b]	159.55 [cd]
VAM	3.51 [b]	72.67 (58.48)[bc]	9.85 [a]	2.68 [a]	715.63 [ab]	194.75 [ab]
P. indica	3.69 [a]	82.00 (64.97)[ab]	9.91 [a]	2.56 [a]	811.92 [a]	209.76 [a]
Carbendazim	3.53 [b]	83.33 (66.14)[a]	9.78 [a]	2.16 [b]	814.61 [a]	180.14 [bc]
Tetracycline	3.51 [b]	72.00 (58.18)[c]	9.15 [ab]	2.48 [a]	682.75 [b]	179.04 [bc]
Control	3.40 [b]	70.00 (56.86)[c]	9.47 [b]	2.05 [b]	640.97 [b]	143.02 [d]
CD (P=0.05)	0.157*	6.66*	0.49*	0.24*	100.43*	27.77**

[$]Different superscript letters indicate significant differences (P<0.05). CD, Critical difference.
**, P<0.01; *, P<0.05. [^]Values in the parenthesis are arcsine transformed values.

to plants by protecting the roots from soil-borne pathogens (Banerjee *et al.* 2023). Chemical seed treatments offer both prophylactic and protective effects, enhancing vigour from the initial stages of crop growth. Vegetative traits like plant establishment at 30 DAP, 60 DAP, plant height was higher in bulbs treated with chemicals. In contrast, the effectiveness of bioagents is typically slower initially, as they require time to multiply and establish. However, once established, repeated applications are seldom needed. Chemicals, on the other hand, are subject to degradation and losses like leaching, necessitating repeated spraying or application.

Effect of bioagents on seed yield and yield contributing characteristics: Onion bulb treatment with bioagents enhanced the seed yield (Fig. 1C). Seed yield is the factor of number of seeds and the test weight. Umbel diameter was significantly higher in *P. indica* treatment (5.01 cm) compared to 4.53 cm in control (Table 1). Bold seeds have high seed vigour and seed quality (Yalamalle V R 2016). 1000-seed weight was significantly higher in *P. indica* treatment (3.69 g), which was 8.53% higher than control. Seed yield/plant was significantly higher in *P. indica* treatment (22.60 g/plant), which was higher in comparison to chemical treatment as well as control. Though the chemical treatments gives higher values for vegetative and some reproductive traits, chemicals are subjected to leaching and degradation in the soil and plant, while bioagents like *P. indica* is known to persist in the root (Fig. 2) through the life cycle of the plant. The enhancement of seed yield by *P. indica* has been previously reported which was attributed to enhanced resistance to biotic and abiotic stress and improved nutrient uptake (Singh *et al.* 2003).

Effect of bioagents on seed quality: Onion bulbs treated with carbendazim reported the highest seed germination (83.33%), and among the bioagents, *P. indica* reported the highest germination (82.00%) (Table 2). The highest seedling length of 9.91 cm was recorded in *P. indica* treatment, while in control the average seedling length was 9.47 cm (Table 2). VAM reported the highest seedling dry weight (2.68 mg/seedling) followed by *P. indica* (2.56 mg/seedling) (Table 2). The field establishment is governed by the seedling vigour, bulb treatment with carbendazim reported highest vigour index-I (814.61), and among bioagents *P. indica* recorded highest vigour index-I (811.92) (Table 2). *P. indica* recorded the highest vigour index-II (209.76), which was 46.67% higher than control (Table 2). The enhanced seed quality in *P. indica* treatment may be attributed to higher test weight (Table 2).

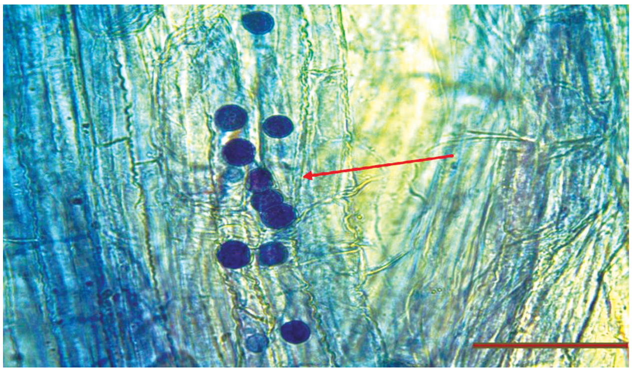

Fig. 2 Microscopic view of *Piriformospora indica* chlamydospores in cortical region of onion root stained with trypan blue after 45 days of treatment.
Scale bar = 20 µm.

SUMMARY

The onion seed crop is affected by several bacterial and fungal rot, which causes lodging, yield, and seed quality losses. In the present study the performance of three biocontrol agents- *Piriformospora indica*, Vesicular arbuscular mycorrhiza (VAM) and *Trichoderma viride* with fungicide and bactericide as a standard control in reducing lodging and enhancing the seed yield and quality in onion was evaluated. The results showed that among the bioagents, *P. indica* recorded the highest bulb germination (98.39%), highest plant stand (87.10%), umbel diameter (5.01 cm), and lowest lodging (27.56%). Whereas VAM recorded the highest plant height (53.53 cm). The performance of *P. indica* was equivalent to standard chemical control carbendazim for several characteristics and even superior in characteristics like–seed yield (22.60 g/plant) and seed quality traits like–1000-seed weight (3.69 g), and seed vigour index-II (209.76). These results suggest that onion bulb

treatment with *P. indica* is beneficial in reducing pesticide use and lodging, and increasing the productivity and seed quality. To the best of our knowledge, this is the first report on the use of *P. indica* in onion seed crop. Onion seed crop is pollinated by insects, primarily by honeybees, which are highly susceptible to pesticide applications. Therefore, onion seed bulb treatment with *P. indica* can be an essential component of integrated crop management in onion seed production for sustainable agriculture.

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