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ORIGINAL ARTICLE

Biofumigation based integrated disease management against *Athelia rolfsii* (syn. *Sclerotium rolfsii* Sacc.) induced collar rot disease of betelvine (*Piper betle* L.)

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Abstract

Collar rot of betelvine (*Piper betle* L.) is an important disease in India, caused by *Athelia rolfsii* (Curzi) C.C. Tu and Kimbr. (syn. *Sclerotium rolfsii* Sacc.) Management of this soil borne pathogen is highly challenging in the shade house (*boroj*) condition, where betelvine is grown as a perennial climber. As betel leaves are consumed raw, application of chemical fungicides is highly restricted to safeguard human health. The present study compared various integrated disease management packages by suitable combination of biofumigation, biocontrol and soil solarization strategies and evaluated the best package at farmers' field condition. The treatment combination of "biofumigation with 0.7 kg m^{-2} green biomass of Indian mustard cv. Pusa Mahak" + "curing of soil by resting for 5 months in the form of heap followed by soil solarization for 30 days" + "biocontrol with 10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain" was found to be the most economical and effective disease management option with highest leaf yield in the experimental plot. This package resulted in 76.82% reduction in collar rot incidence, 29.94% increase in leaf yield and 41.45% increase in net income during March-June crop cycle, in farmers' field condition, when compared to the Farmers' Practice (soil drench with 4 L m^{-2} 0.25% Blitox 50 W). *Trichoderma* was found to be highly tolerant to the biofumigation volatiles, which maintained a good soil population (32.78×10^3 CFU g^{-1} soil) in the farmers' plots adopting integrated disease management. Biofumigation with Indian mustard and biocontrol with local isolate of *Trichoderma* offered an economical management of the collar rot disease in betelvine, without compromising the crop yield and the population of *Trichoderma* spp. in soil.

Keywords Betelvine · Collar rot · Biofumigation · Indian mustard · Integrated disease management · *Trichoderma*

Introduction

Betelvine (*Piper betle* L.) is a perennial, evergreen climber, grown for its heart shaped leaves that are widely used as masticatory and traditional medicinal preparations in South-East Asia. The coastal saline zone of West Bengal in India is famous for growing Mitha Pata cultivar of this crop, which is admired for its fennel-like fragrance, sweet taste and low fibre content. The shade loving vines are grown inside a specially constructed shade house, known as *boroj*. However, because of its proneness to several diseases, aggravated by the moist and humid microclimate inside the *boroj*, cultivation of betelvine is highly risky (Sengupta et al. 2011). Among various diseases, it is very difficult to manage and eradicate the collar rot disease, caused by *Athelia rolfsii* (Curzi) C.C. Tu and Kimbr. (syn. *Sclerotium rolfsii* Sacc.). During summer season (April – June), it infects the collar region of the vines, causing rapid wilting and resulting in 17–90% crop loss (Maiti and Sen 1982; Garain et al. 2020).

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Inside the *boroj*, the vines are trailed over individual supports (jute stick), fixed in the ground at upright position. As the matured leaves are plucked periodically, the vines continue to grow to finally reach the roof of the *boroj*. Then, the vines are lowered on the ground and covered with a layer of soil, leaving the apical shoot with 2–3 young leaves to grow further. This "lowering of vines" and burying them with soil is a routine and unique cultural operation in betelvine cultivation that is performed three to four times in a year. The soil, used for this purpose, is collected from the surrounding agricultural fields and stored near the *boroj*. The soil borne pathogens, like *Athelia rolfsii,* often take entry into the *boroj,* along with this untreated soil.

Management of such a soil borne pathogen in the closed shade house condition (*boroj*) is highly challenging. Over the last few decades, soil fumigation with synthetic chemicals (metam-sodium, dazomet, methyl bromide, pentachloronitrobenzene) has been the most widely used method of soil borne disease control but certainly at the cost of several negative attributes like, high volatility, toxicity and carcinogenicity (Baker et al. 1996). Methyl bromide was found to contribute to stratospheric ozone depletion (Van den Berg et al. 1994) and hence was targeted for phase-out by 2005 from the industrialized countries and by 2015 from the developing countries, during the 9th meeting of Montreal Protocol in 1997 (Stapleton et al. 2000; Gullino et al. 2003). In addition to environmental safety, there is also a growing concern of fungicide residues. Since, green betel leaves are chewed raw, considerable emphasis is warranted for a less persistent and more eco-friendly means of managing betelvine diseases.

To reduce such toxic hazards to the environment and human beings, a bio-intensive integrated disease management approach would be an ideal option. Soil solarization has been found effective for managing *A. rolfsii* induced collar rot in betelvine (Deshpande and Tiwari 1991). The use of biocontrol agents like *Trichoderma* spp., is widely popularized in betelvine cultivation (Datta et al. 2011). But the use of native isolates of such fungal antagonists has been less explored in the problematic saline soils (17.5%) of the coastal saline zone of West Bengal.

Biofumigation is another biological approach where plant materials are incorporated into the soil to control soil borne pathogens through the release of toxic volatiles (Kirkegaard and Sarwar 1998). Several cruciferous plants contain high quantities of glucosinolates, which in presence of an endogenous enzyme myrosinase, get hydrolysed to form isothiocyanates, thiocyanates, nitriles and oxa-zolidinethiones that are highly biocidal to many fungi, bacteria, nematodes and insects (Sarwar et al. 1998). Soil incorporation of chopped biomass of Indian mustard (*Brassica juncea* L.) has been found to produce allyl isothiocyanate upto a concentration of 100 nmol g^{-1} soil (Matthiessen and Kirkegaard 2006) that could effectively suppress the mycelial growth of *A. rolfsii* (Harvey et al. 2002).

In the present investigation, a comprehensive effort has been made to develop an integrated disease management package against the collar rot disease of betelvine, combining the use of biofumigation, native isolate of biocontrol agent and soil solarization.

Materials and methods

Experimental site and cultural conditions

To compare different integrated disease management treatments, an experiment was conducted during 2016 and 2017 at Sagar block, in the coastal saline zone of West Bengal, India. A six-year-old betelvine *boroj* (latitude 21°43′59.49"N, longitude 88°07′17.67"E), grown with Mitha Pata cultivar, was selected for the experiment, considering high collar rot disease incidence during the previous years (31% and 34% in 2014 and 2015, respectively). The vines were spaced at 60 cm (row-to-row) and 15 cm (plant-to-plant). Manuring, irrigation and other cultural operations were carried out as per the recommended package of practice. Based on the time of lowering the vines (March, July and October), the crop season was divided into three cycles (March to June, July to September and October to February). Considering the seasonal incidence of the collar rot disease (Garain et al. 2020), the experiment was planned to target the March-June crop cycle (Table 1).

Design of experiment and components of the integrated disease management treatments

To evaluate the effect of different levels of biofumigation, curing of soil and disease control, the treatments were arranged using split-split-plot design in three replications (supplementary data). The experimental plot (*boroj*) was divided into three blocks and each of them was used as one replication. Each block was divided into two main plots, each main plot into two sub-plots and each sub-plot into four sub-sub-plots. The sub-sub-plot was of 7.5 m × 1.2 m size, containing 100 vines in two rows. The main and sub-plots included two levels of biofumigation ('without biofumigation' and 'biofumigation') and two levels of curing of soil ('without curing of soil' and 'curing of soil'), respectively. The sub-sub-plots were assigned with four levels of disease control (a. no treatment as 'control'; b. application of 4 L m^{-2} 0.25% Blitox 50 W as 'chemical fungicide'; c. application of 10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain as 'biocontrol agent' and d. combined application

Table 1 Timeline of different treatments

Year	Curing of the soil	Sowing of biofumigant crop (Indian mustard cv. Pusa Mahak)	Lowering of vine	Soil incorporation of biofumigant crop	Soil amendment with *Trichoderma* sp. T-Nam colonized whole rice grain	Covering the lowered vines with soil	Soil drenching with Blitox 50 W fungicide
2016	1.10.2015 to 21.3.2016	27.1.2016	20.03.2016	22.3.2016 to 23.3.2016	22.3.2016	22.3.2016 to 23.3.2016	28.3.2016
2017	3.10.2016 to 23.3.2017	29.1.2017	24.03.2017	25.3.2017 to 26.3.2017	25.3.2017	25.3.2017 to 26.3.2017	1.4.2017

of '10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain + 4 L m^{-2} 0.25% Blitox 50 W').

For biofumigation, Pusa Mahak cultivar of Indian mustard (*B. juncea*) was selected. The biofumigation potential and the effective dose of biofumigation of the cultivar were evaluated in a previous experiment (Garain et al. 2021). The seeds were sown (3 kg ha^{-1}) on the biofumigation plots as an intercrop within the standing crop of betelvine, during January (Table 1). The mustard crop was harvested manually in March, at pre-flowering stage and the finely chopped green biomass was spread over the soil at the rate of 0.7 kg m^{-2}. At the same time, the remaining matured betel leaves were harvested and the vines were lowered on the ground by coiling. The lowered vines and the mustard biomass were then covered with a 5-cm layer of soil.

The soil, used for covering the vines after lowering, was either untreated (for sub-plot 'without curing of soil') or subjected to special curing (for sub-plot 'curing of soil'). The top soil was collected during October, from the nearby agricultural field (following a cropping system of "brinjal – beans – cucumber" for past two years) and was rested for five months as a heap (2 m height) outside the *boroj*. Then the soil was spread on the ground (10-cm layer), moistened and covered with transparent polythene sheet (25 µm) for 30 days, under the sun for soil solarization (Table 1).

The fungicide Blitox 50 W (manufactured by Tata Rallis India Ltd.), containing copper oxychloride 50% WP (w/w) as active ingredient, was selected due to its label claim for use in betelvine in India as per the recommendation of the Central Insecticide Board and Registration Committee (Anonymous 2020). The respective sub-sub-plots were drenched with 4 L m^{-2} 0.25% Blitox 50 W, after lowering of vines, in the month of March (Table 1).

Trichoderma sp. T-Nam, previously isolated from the rhizosphere of betelvine on an improved *Trichoderma* Selective Medium (Elad and Chet 1983), was used as the biocontrol agent. Its antagonistic potential against *A. rolfsii* was confirmed (unpublished data). The *Trichoderma* culture was first mass multiplied on sterilized whole rice grains and stored at 4 °C. Then, 10 g of *Trichoderma* colonized whole rice grain was mixed with 1 kg vermicompost and applied m^{-2} area, in the respective sub-sub-plots, after lowering the vines in March (Table 1). The number of spores produced by *Trichoderma* colonized whole rice grain was counted with the help of a haemocytometer chamber and found to be in the range of 3.14×10^9 to 6.96×10^9 g^{-1} dry matter.

Disease incidence

Number of vines infected by *A. rolfsii* and showing collar rot symptoms (rapid wilting with water soaked lesion at collar region followed by visible presence of white ropy mycelium and sclerotia) were counted out of the 100 vines in each plot, during March to June crop cycle. The disease incidence (DI %) was then recorded as percentage of vines infected by *A. rolfsii*.

Yield parameters

As the vines began to grow, they were again trailed over the perpendicular support. Harvesting of matured leaves started from the middle of April and continued up to the end of June when the vines were lowered for the second time. Number of leaves harvested during March to June from each plot was recorded and extrapolated to express as "10^5 leaves ha^{-1}". To determine leaf thickness, 10 leaves were collected from each treatment. Circular discs were cut from each leaf by pressing a metal cap against the leaves. Then all the leaf discs were placed one over another and the total thickness was measured with a Vernier calliper scale. From this, average thickness of one leaf was calculated. For fresh weight calculation, 100 leaves were randomly collected from each treatment, weighed with the help of a digital balance and expressed as "fresh weight of 100 leaves".

Economic analysis

The total cost of cultivation was calculated including the cost of manuring, inter-culture operations, irrigation, weeding, minor repairing, harvesting and the specific cost for a particular treatment. Gross and net incomes were calculated based on the average market price of betel leaves. Net income was

calculated by subtracting the total cost of cultivation from gross income. Then the "cost of treatment" and "additional net income" were calculated for each individual treatment, by deducting the total cost and net income of control plot ("without biofumigation" + "without curing of soil" + "control" combination) from the total cost and net income of a particular treatment plot, respectively. All prices were extrapolated and expressed as "Indian Rupees per ha (₹ ha^{-1})", according to the 2020 market rate. The "Benefit–Cost Ratio (BCR)" was calculated as a ratio between gross income and total cost and expressed as the amount of gross income for each rupee spent.

Validation of best performing integrated disease management package in farmers' field

The best performing integrated disease management combination, selected on the basis of the two years experiment, was demonstrated in farmers' field for two years (2018 and 2019) with 13 replications. The *boroj* were selected considering the high level of collar rot incidence (20% to 22%) over the past two years (2016 and 2017). Each of the selected farmers had 200 m^2 *boroj* of similar age (five year old), growing same cultivar (Mitha Pata) and following uniform agronomical practices (nutrient management, irrigation schedule, etc.). Each *boroj* was divided into two halves of 100 m^2 plot. In one half, Farmer's Practice was followed where the soil was drenched by 0.25% Blitox 50 W at the rate of 4 L m^{-2}, after lowering of vines during March. In the other half, the selected integrated disease management combination was followed. The disease incidence (DI %) and yield were recorded and the economic analysis was calculated for the March to June crop cycle, as discussed earlier.

Enumeration of *Trichoderma* population in the farmers' field

The population of *Trichoderma* in soil was enumerated by serial dilution plating on the improved *Trichoderma* selective media (Elad and Chet 1983). Soil samples from both the plots ("farmer's practice" and "integrated disease management") were collected after the final treatment, at monthly intervals (15th of April, 15th of May and 15th of June) during 2018 and 2019. The samples were air dried under shade, ground into fine powder and mixed properly. A stock solution (10^{-1} dilution) was prepared by dissolving 100 g soil into 900 ml sterile distilled water. From this solution serial dilution of samples was prepared up to 10^{-5} dilution. One ml soil–water suspension from each diluted samples was then poured into sterilized Petri plate where 20 ml of the *Trichoderma* selective media was added. The plates were incubated in BOD incubator at 28 ± 1^0C for 4 days and the *Trichoderma* colonies were counted. The *Trichoderma* population was expressed as Colony Forming Unit (CFU) per gram of soil.

Statistical analysis

All statistical analysis was performed for the pooled data of the respective experiments with IBM's SPSS Statistics 20. Shapiro–Wilk test was used for assessing the null hypothesis that the data of disease incidence, yield and economic parameters were normally distributed. Angular transformation was applied to normalise the data of disease incidence (values in percentage). After fulfilling the normality of data, the analysis of variance (ANOVA) and multiple comparisons were performed under split-split-plot design. The means of the treatments were compared based on LSD values and the significant differences were determined at $p = 0.05$.

Results

Disease incidence

The impact of different levels of biofumigation, curing of soil and disease control on collar rot disease incidence, yield and economic parameters was evaluated based on the pooled data of 2016 and 2017. Variance analysis of disease incidence (Table 2) revealed a significant difference between the two levels of biofumigation ($p = 0.008$), two levels of curing of soil ($p = 0.001$)) and four levels of disease control ($p < 0.001$). Mean comparison revealed that disease incidence was significantly lower at "biofumigation" plots than at "without biofumigation" (Fig. 1). Similarly, the mean disease incidence in "curing of soil" was significantly lower than "without curing of soil". Mean comparison of disease incidence divided different levels of disease control into three groups (a. "control", b. "Blitox 50 W" and c. "*Trichoderma* sp. T-Nam" and "*Trichoderma* sp. T-Nam + Blitox 50 W"). The disease control levels "*Trichoderma* sp. T-Nam" and "*Trichoderma* sp. T-Nam + Blitox 50 W" recorded lowest disease incidences, which were statistically at par, but both differed from the "control" and "Blitox 50 W" ($p = 0.05$).

None of the interactions "biofumigation" × "curing of soil" or "biofumigation" × "disease control" or "curing of soil" × "disease control" had any significant effect on the disease incidence ($p > 0.05$). However, when all the three factors were considered together (Table 2), the interaction ("biofumigation" × "curing of soil" × "disease control") was found to be significant ($p = 0.027$). As per the pooled data, the least disease incidence was recorded in the treatment combination of "biofumigation + curing of soil + *Trichoderma* sp. T-Nam and Blitox 50 W" ($4.67 \pm 0.73\%$), which

Table 2 Disease incidence and yield parameters (pooled data of 2016 and 2017 for March to June crop cycle)

Main plot (Biofumigation)	Subplot (Curing of soil)	Sub subplot (Disease control)	Disease Incidence (%)	Yield ($\times 10^5$ leaves ha^{-1})	Leaf thickness (mm)	Fresh leaf weight (g 100 leaves^{-1})
Without biofumigation	Without curing of soil	Control	33.00 ± 2.75^{a}	20.83 ± 0.13^{a}	0.523 ± 0.004	297.92 ± 9.05
		4 L m^{-2} 0.25% Blitox 50 W	28.00 ± 1.32^{b}	22.28 ± 0.11^{c}	0.527 ± 0.007	299.53 ± 12.50
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain	16.67 ± 1.09^{e}	25.80 ± 0.07^{h}	0.512 ± 0.007	300.98 ± 8.89
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain + 4L m^{-2} 0.25% Blitox 50 W	15.83 ± 1.45^{e}	26.04 ± 0.09^{i}	0.518 ± 0.006	302.68 ± 9.69
	Curing of soil	Control	29.00 ± 2.47^{b}	22.00 ± 0.09^{b}	0.518 ± 0.003	297.92 ± 9.11
		4 L m^{-2} 0.25% Blitox 50 W	24.67 ± 2.80^{c}	23.26 ± 0.12^{d}	0.525 ± 0.006	299.47 ± 12.52
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain	10.67 ± 1.17^{g}	27.69 ± 0.09^{k}	0.533 ± 0.019	300.82 ± 8.94
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain + 4L m^{-2} 0.25% Blitox 50 W	10.17 ± 1.20^{g}	27.84 ± 0.11^{l}	0.527 ± 0.002	302.45 ± 9.82
Biofumigation "with 0.7 kg m^{-2} Indian mustard green biomass"	Without curing of soil	Control	23.67 ± 0.73^{c}	23.68 ± 0.08^{e}	0.518 ± 0.006	295.65 ± 8.33
		4 L m^{-2} 0.25% Blitox 50 W	20.83 ± 1.33^{d}	24.59 ± 0.10^{f}	0.522 ± 0.009	297.42 ± 9.14
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain	8.67 ± 0.88^{h}	28.38 ± 0.09^{m}	0.518 ± 0.016	301.88 ± 9.97
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain + 4L m^{-2} 0.25% Blitox 50 W	8.50 ± 0.76^{h}	28.35 ± 0.07^{m}	0.525 ± 0.005	303.08 ± 9.82
	Curing of soil	Control	18.00 ± 1.32^{e}	25.42 ± 0.07^{g}	0.518 ± 0.012	296.85 ± 10.14
		4 L m^{-2} 0.25% Blitox 50 W	13.83 ± 0.67^{f}	26.66 ± 0.05^{j}	0.528 ± 0.011	297.00 ± 6.90
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain	4.83 ± 0.73^{i}	29.56 ± 0.08^{o}	0.527 ± 0.006	301.52 ± 11.37
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain + 4L m^{-2} 0.25% Blitox 50 W	4.67 ± 0.73^{i}	29.44 ± 0.06^{n}	0.520 ± 0.003	305.78 ± 9.66
Biofumigation			$F_{(1,24)} = 127.49^{**}$ ($p = 0.008$)	$F_{(1,24)} = 9138.76^{**}$ ($p < 0.001$)	$F_{(1,24)} = 0.31^{NS}$ ($p = 0.63$)	$F_{(1,24)} = 0.11^{NS}$ ($p = 0.767$)
Curing of soil			$F_{(1,24)} = 99.53^{**}$ ($p = 0.001$)	$F_{(1,24)} = 21{,}010.49^{**}$ ($p < 0.001$)	$F_{(1,24)} = 10.29^{NS}$ ($p = 0.33$)	$F_{(1,24)} = 0.20^{NS}$ ($p = 0.674$)
Biofumigation x Curing of soil			$F_{(1,24)} = 1.24^{NS}$ ($p = 0.328$)	$F_{(1,24)} = 9.47^{NS}$ ($p = 0.37$)	$F_{(1,24)} = 1.14^{NS}$ ($p = 0.35$)	$F_{(1,24)} = 0.37^{NS}$ ($p = 0.574$)
Disease control			$F_{(3,24)} = 802.35^{**}$ ($p < 0.001$)	$F_{(3,24)} = 48{,}739.71^{**}$ ($p < 0.001$)	$F_{(3,24)} = 0.40^{NS}$ ($p = 0.75$)	$F_{(3,24)} = 8.98^{**}$ ($p < 0.001$)
Biofumigation x Disease control			$F_{(3,24)} = 0.21^{NS}$ ($p = 0.886$)	$F_{(3,24)} = 564.79^{**}$ ($p < 0.001$)	$F_{(3,24)} = 0.15^{NS}$ ($p = 0.93$)	$F_{(3,24)} = 1.05^{NS}$ ($p = 0.390$)
Curing of soil x Disease control			$F_{(3,24)} = 2.54^{NS}$ ($p = 0.080$)	$F_{(3,24)} = 4.56^{*}$ ($p = 0.011$)	$F_{(3,24)} = 0.83^{NS}$ ($p = 0.49$)	$F_{(3,24)} = 0.14^{NS}$ ($p = 0.935$)
Biofumigation x Curing of soil x Disease control			$F_{(3,24)} = 3.63^{*}$ ($p = 0.027$)	$F_{(3,24)} = 399.84^{**}$ ($p < 0.001$)	$F_{(3,24)} = 0.40^{NS}$ ($p = 0.75$)	$F_{(3,24)} = 0.16^{NS}$ ($p = 0.925$)

Data are represented as mean of three replication ± standard error, * Significant difference at $p = 0.05$, ** Significant difference at $p = 0.01$, ^{NS} Not significant at $p > 0.05$,

Figures superscripted with different letters in a column are significantly different as per LSD at $p = 0.05$

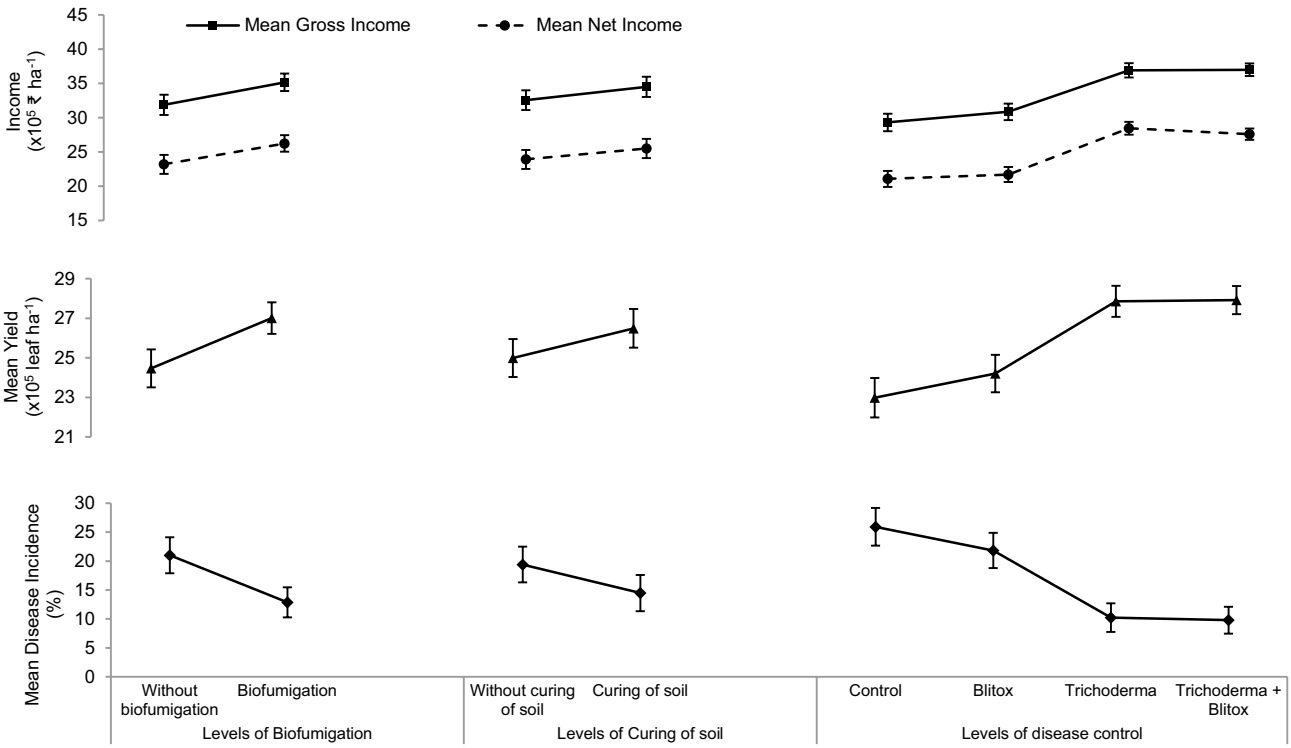

Fig. 1 Mean comparison of disease incidence, yield and income at different levels of biofumigation, curing of soil and disease control (pooled data of 2016 and 2017); the error bars represent standard errors of the data

was statistically at par with "biofumigation + curing of soil + *Trichoderma* sp. T-Nam" (4.83 ± 0.73%).

Yield parameters

Variance analysis of leaf yield (Table 2) showed a significant difference between the two levels of biofumigation ($p < 0.001$), two levels of curing of soil ($p < 0.001$)) and four levels of disease control ($p < 0.001$). Mean comparison revealed that yield was significantly higher with "biofumigation" than "without biofumigation" (Fig. 1). Similarly, the mean leaf yield in "curing of soil" was significantly higher than "without curing of soil". The mean comparison of leaf yield divided the four levels of disease control into three groups (a. "control", b. "Blitox 50 W" and c. "*Trichoderma* sp. T-Nam" and "*Trichoderma* sp. T-Nam + Blitox 50 W"). The disease control levels "*Trichoderma* sp. T-Nam" and "*Trichoderma* sp. T-Nam + Blitox 50 W" gave higher yield, which were statistically at par but both differed significantly ($p = 0.05$) from the "control" and "Blitox 50 W".

Except the "biofumigation" × "curing of soil" ($p > 0.05$), all other treatment interactions, "biofumigation" × "disease control" ($p < 0.001$), "curing of soil" × "disease control" ($p = 0.011$) and "biofumigation" × "curing of soil" × "disease control" ($p < 0.001$) were found to be significant (Table 2). As per the pooled data, the highest yield (29.56×10^5 leaves ha^{-1}) was obtained in the treatment combination of "biofumigation + curing of soil + *Trichoderma* sp. T-Nam", which was followed by "biofumigation + curing of soil + *Trichoderma* sp. T-Nam and Blitox 50 W" (29.44×10^5 leaves ha^{-1}). Lowest yield was recorded in "without biofumigation + without curing of soil + control" treatment combination (20.83×10^5 leaves ha^{-1}).

None of the treatments with different levels of biofumigation, curing of soil and disease control or their interactions had any significant ($p > 0.05$) influence on thickness of betel leaves (Table 2). The biofumigation and curing of soil also had no significant ($p > 0.05$) influence on fresh weight of leaves. However, a significant difference was observed between the levels of disease control ($p < 0.001$), on fresh weight of leaves. The mean comparison of fresh leaf weight divided the four levels of disease control into two groups (a. "control" and "Blitox 50 W" and b. "*Trichoderma* sp. T-Nam" and "*Trichoderma* sp. T-Nam + Blitox 50 W"). The disease control levels "*Trichoderma* sp. T-Nam" and "*Trichoderma* sp. T-Nam + Blitox 50 W" resulted in significantly ($p = 0.05$) higher fresh weight of leaves than the other group.

Economic parameters

The average market price of betel leaf was ₹ 1.25 and ₹ 1.30 per leaf during 2016 and 2017, respectively. But the leaves from plots treated with *Trichoderma* sp. T-Nam fetched a better price (₹ 1.30 and ₹ 1.35 during 2016 and 2017, respectively) due to their superior quality in terms of colour, lustre, texture, shape and storability.

Both, the gross and net income varied significantly ($p < 0.001$) at different levels of "biofumigation", "curing of soil" and "disease control" (Table 3). Mean comparison revealed that income was significantly higher with "biofumigation" than "without biofumigation" (Fig. 1). Similarly, the means of gross and net income in "curing of soil" were significantly higher than "without curing of soil". The mean income was also significantly different at the four levels of disease control. Though gross income was highest in the disease control level with "*Trichoderma* sp. T-Nam + Blitox 50 W", the net income was highest in "*Trichoderma* sp. T-Nam". Except the "biofumigation" × "curing of soil" ($p > 0.05$) interaction, all other treatment interactions, *i.e.*, "biofumigation" × "disease control" ($p < 0.001$), "curing of soil" × "disease control" ($p < 0.01$) and "biofumigation" × "curing of soil" × "disease control" ($p < 0.001$), had significant impact on gross and net income (Table 3). As per the pooled data, the highest gross income (₹ 3,917,130.00 ha^{-1}) and net income (₹ 3,042,130.00 ha^{-1}) was obtained in the treatment combination of "biofumigation + curing of soil + *Trichoderma* sp. T-Nam", for the March-June crop cycle.

Highest benefit–cost ratio (4.48) was obtained under the treatment combinations "biofumigation + curing of soil + *Trichoderma* sp. T-Nam" and "biofumigation + without curing of soil + *Trichoderma* sp. T-Nam" (Table 3). The relative cost of individual disease management treatments from highest to lowest order was found as: "application of Blitox 50 W" (₹ 93,667.00 ha^{-1}) > "curing of soil" (₹ 35,333.00 ha^{-1}) > "biofumigation" (₹ 30,667.00 ha^{-1}) > "application of *Trichoderma* sp. T-Nam" (₹ 23,667.00 ha^{-1})'. Highest additional yield (8.73×10^5 leaves ha^{-1}) and additional net return (₹ 1,179,130.00 ha^{-1}) was obtained in the "biofumigation + curing of soil + *Trichoderma* sp. T-Nam" treatment combination.

Performance of integrated disease management package in farmers' field

Considering the lowest collar rot incidence, highest yield and maximum profit during the two years experiment, the best performing integrated disease management (IDM) package was found to be the combination of "biofumigation with 0.7 kg m^{-2} green biomass of Indian mustard cv. Pusa Mahak" + "curing of soil by resting for 5 months in the form of heap followed by soil solarization for 30 days" + "biocontrol with 10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain".

The data of disease incidence, yield and economic parameters showed significant ($p = 0.05$) difference between the two treatments (Table 4). The IDM package resulted in reduction in collar rot incidence by 76.82%, increase in leaf yield by 29.94% and increase in net income by 41.45%, over the "farmer's practice". The benefit–cost ratio was 5.53 in the IDM plots, compared to 4.52 in the farmer's practice plots. The population of *Trichoderma* spp. in the soil was consistently and significantly ($p = 0.05$) higher in the plots following the IDM package than in the farmer's practice plots, throughout the crop cycle.

Discussion

Biofumigation with Indian mustard significantly reduced collar rot disease incidence in betelvine. Soil incorporation of mustard biomass in the biofumigated plot may have negatively impacted the growth and development of *A. rolfsii* in the soil system. Stapleton and Duncan (1998) recorded 87–100% reduction in sclerotial germination in *A. rolfsi* through soil ammendment with fresh and dried crop residue of cruciferous plants. Indian mustard has also been reported to effectively suppress the mycelial growth of *A. rolfsii* (Harvey et al. 2002) and reduce stem rot disease in groundnut (Yella Goud et al. 2017) under field condition. Biofumigation with *Brassica* crops has been successfully demonstrated by many researchers to control other soil borne pathogens like, *Rhizoctonia solani, Pythium* spp., *Fusarium* spp., *Sclerotinia sclerotium,* etc. (Charron and Sams 1999; Relevante and Cumagun 2013; Baysal-Gurel et al. 2019).

The 'curing of soil' treatment consisted of two parts, firstly, resting the soil for five months outside the *boroj* in the form of a soil heap of 2 m height and secondly, exposing the soil to solar heat (soil solarization) before its application inside the *boroj*. Resting the soil in the form of a heap may have helped to decrease the viability of the sclerotia by limiting oxygen for respiration or through the effect of physical pressure on sclerotia at greater depths resulting in reduction of sclerotial germination (Punja and Jenkins 1984). Soil solarization has also been effectively utilized for managing *A. rolfsii* induced collar rot in betelvine (Deshpande and Tiwari 1991). Heating of sclerotia (*A. rolfsii*) in natural soil allows organic substances to leak from the sclerotia that apparently stimulate its extensive colonization by beneficial soil microorganisms (Lifshitz et al. 1983). All of these developments may have apparently weakened the sclerotia and finally reduced their inoculum potential and strongly promoted their colonization by soil microorganisms. Hence, pre-treatment of the soil before its application to the plant

Table 3 Economic analysis of the pooled data of 2016 and 2017 for March to June crop cycle (all prices are in ₹ ha^{-1})

Main plot (Biofumigation)	Subplot (Curing of soil)	Sub subplot (Disease control)	Gross Income	Net Income	BCR[a]	Cost of treatment	Additional yield ($\times 10^5$ leaves ha^{-1})	Additional net income
Without biofumigation	Without curing of soil	Control	2,656,444.00[a]	1,863,111.00[a]	3.35	-	-	-
		4 L m^{-2} 0.25% Blitox 50 W	2,841,463.00[c]	1,954,796.00[b]	3.20	93,667.00	1.45	91,796.00
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain	3,418,537.00[i]	2,601,870.00[j]	4.19	23,667.00	4.97	738,870.00
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain + 4L m^{-2} 0.25% Blitox 50 W	3,451,278.00[j]	2,541,278.00[i]	3.79	117,000.00	5.21	678,278.00
	Curing of soil	Control	2,806,046.00[b]	1,977,713.00[c]	3.39	35,333.00	1.17	114,713.00
		4 L m^{-2} 0.25% Blitox 50 W	2,965,583.00[d]	2,043,917.00[d]	3.22	128,667.00	2.43	180,917.00
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain	3,669,981.00[k]	2,818,315.00[l]	4.31	58,667.00	6.86	955,315.00
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain + 4L m^{-2} 0.25% Blitox 50 W	3,689,657.00[l]	2,744,657.00[k]	3.90	152,000.00	7.01	881,657.00
Biofumigation "with 0.7 kg m^{-2} Indian mustard green biomass"	Without curing of soil	Control	3,019,491.00[e]	2,195,824.00[e]	3.67	30,667.00	2.85	332,824.00
		4 L m^{-2} 0.25% Blitox 50 W	3,135,750.00[f]	2,225,750.00[f]	3.45	117,000.00	3.76	362,750.00
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain	3,760,519.00[m]	2,920,519.00[m]	4.48	47,000.00	7.55	1,057,519.00
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain + 4L m^{-2} 0.25% Blitox 50 W	3,756,639.00[m]	2,823,306.00[l]	4.02	140,333.00	7.52	960,306.00
	Curing of soil	Control	3,241,222.00[g]	2,389,556.00[g]	3.81	58,667.00	4.59	526,556.00
		4 L m^{-2} 0.25% Blitox 50 W	3,400,194.00[h]	2,455,194.00[h]	3.60	152,000.00	5.83	592,194.00

Table 3 (continued)

Main plot (Biofumigation)	Subplot (Curing of soil)	Sub subplot (Disease control)	Gross Income	Net Income	BCR[a]	Cost of treatment	Additional yield ($\times 10^5$ leaves ha^{-1})	Additional net income
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain	3,917,130.00^{o}	3,042,130.00^{o}	4.48	82,000.00	8.73	1,179,130.00
		10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain + 4L m^{-2} 0.25% Blitox 50 W	3,901,130.00^{n}	2,932,796.00^{n}	4.03	175,333.00	8.61	1,069,796.00
Biofumigation			$F_{(1,24)} = 9593.44^{**}$ ($p < 0.001$)	$F_{(1,24)} = 8233.43^{**}$ ($p < 0.001$)				
Curing of soil			$F_{(1,24)} = 20{,}886.39^{**}$ ($p < 0.001$)	$F_{(1,24)} = 14{,}177.71^{**}$ ($p < 0.001$)				
Biofumigation x Curing of soil			$F_{(1,24)} = 4.89^{NS}$ ($p = 0.091$)	$F_{(1,24)} = 8.20^{NS}$ ($p = 0.46$)				
Disease control			$F_{(3,24)} = 83{,}377.81^{**}$ ($p < 0.001$)	$F_{(3,24)} = 77{,}048.57^{**}$ ($p < 0.001$)				
Biofumigation x Disease control			$F_{(3,24)} = 530.93^{**}$ ($p < 0.001$)	$F_{(3,24)} = 510.25^{**}$ ($p < 0.001$)				
Curing of soil x Disease control			$F_{(3,24)} = 7.60^{**}$ ($p = 0.001$)	$F_{(3,24)} = 5.53^{**}$ ($p = 0.005$)				
Biofumigation x Curing of soil x Disease control			$F_{(3,24)} = 457.81^{**}$ ($p < 0.001$)	$F_{(3,24)} = 468.16^{**}$ ($p < 0.001$)				

Figures superscripted with different letters in a column are significantly different as per LSD at $p = 0.05$

[a]Benefit cost ratio = Gross income ÷ Total cost,* Significant difference at $p = 0.05$, ** Significant difference at $p = 0.01$, ^{NS} Not significant at $p > 0.05$

Table 4 Result of demonstration of the integrated disease management package over farmer's practice (pooled data of 2018 and 2019 for March to June crop cycle)

Treatment	Collar rot incidence (%)	Yield ($\times 10^5$ leaves ha^{-1})	Net income (₹ ha^{-1})	Benefit—Cost Ratio	*Trichoderma* population in soil ($\times 10^3$ CFU g^{-1} soil)		
					April	May	June
FP	17.90 ± 0.42^a	25.45 ± 0.13^a	$2,823,139.00 \pm 18230^a$	4.52 ± 0.02^a	2.95 ± 0.11^a	3.18 ± 0.11^a	3.26 ± 0.13^a
IDM	4.15 ± 0.25^b	33.07 ± 0.23^b	$3,993,193.00 \pm 36173^b$	5.53 ± 0.08^b	19.71 ± 0.45^b	28.34 ± 0.62^b	32.78 ± 0.57^b
SEm (±)	0.31	0.23	34,387.16	0.05	0.33	0.45	0.42
CD (p = 0.05)	0.96	0.71	148,544.68	0.17	1.02	1.38	1.30
% change in IDM over FP	-76.82%	29.94%	41.45%	-	568.14%	791.20%	905.52%

Data are represented as mean of thirteen replications ± standard error. Figures superscripted with different letters are significantly different based on LSD at $p = 0.05$

FP = Farmer's Practice (4L m^{-2} 0.25% Blitox 50 W fungicide)

IDM = Integrated disease management (biofumigation with 0.7 kg m^{-2} green biomass of Indian mustard cv. Pusa Mahak + curing of soil by resting the soil for 5 months in the form of heap followed by soil solarization for 30 days + biocontrol with 10 g m^{-2} *Trichoderma* sp. T-Nam colonized whole rice grain)

base eventually helped to restrict the entry of the soil borne inoculum of *A. rolfsii,* inside the *boroj*.

Application of the native isolate, *Trichoderma* sp. T-Nam, was also superior in reducing collar rot incidence, either alone or in combination with Blitox 50 W fungicide, over the "control" and "Blitox 50 W". Since betel leaves are consumed as raw, application of chemical fungicides to this crop is highly restricted. In India, only copper based contact fungicides (like copper oxychloride) are permitted for use in betelvine (Anonymous 2020). But, copper oxychloride (Blitox 50 W) alone could not provide satisfactory control against collar rot disease in betelvine. The presence of melanin might have imparted resistance to digestion of the sclerotia of *A. rolfsii* by chemical agents (Bloemofield and Alexander 1967). However, *Trichoderma* spp. has been proved to penetrate the highly melanized walls of sclerotia and degrade them completely (Elad and Mishagi 1985), which is not possible by using chemical fungicides. Application of *Trichoderma harzianum* has also been found to increase leaf yield in betelvine (Singh and Singh 2005), apart from successful control of collar rot disease (Datta et al. 2011). The increase in leaf yield and improvement of leaf quality, observed in the *Trichoderma* sp. T-Nam treated plots in our experiment corroborates with the previous findings. The *Trichoderma* can also be mixed with the cured soil directly before covering the lowered vines, for its uniform distribution in the *boroj*.

The combined application of "biofumigation + curing of soil + *Trichoderma* sp. T-Nam" as well as the "biofumigation + curing of soil + *Trichoderma* sp. T-Nam and Blitox 50 W" combination resulted in lowest disease incidence and highest leaf yield. However, the net income was significantly higher in "biofumigation + curing of soil + *Trichoderma* sp. T-Nam" due to the lower cost of treatment. Management of soil borne pathogens by soil drenching with chemical fungicides may be highly effective but a costly affair (Tripathi and Grover 1978), thus increasing overall cost of production. Application of *Trichoderma* has been reported to result in better economic return than chemical control of foot rot disease in betelvine (Dasgupta et al. 2011). Effective use of integrated disease management against collar rot in betelvine has been previously reported by several researchers (Anonymous 2015; Tripathi 2015), where biocontrol agents like the *Trichoderma* spp., organic manures like mustard oil cake and farm yard manure, soil drenching with chemical fungicides and balanced dose of fertilizers have been used in various combinations. There is also similar report of combined use of biofumigation along with *T. harzianum* and *Pseudomonas fluorescens*, which gave effective control against *Rhizoctonia solani* f sp. *sasakii* in Maize (Madhavi and Uma Devi 2018). However, the present study is the first of its kind in using biofumigation with Indian mustard in combination with soil solarization and native isolate of *Trichoderma*, in managing *A. rolfsii* induced collar rot disease of betelvine at field level.

Biofumigation did not show any negative effect on the population of *Trichoderma* in soil. Unlike *A. rolfsii*, the *Trichoderma* spp. is less sensitive to the biofumigation volatiles (Galletti et al. 2008; Garain et al. 2021). The extra biomass or food base, provided during the soil incorporation of a biofumigant crop, helps in multiplication of soil microbes (Omirou et al. 2010). The application of *Trichoderma* sp. T-Nam as well as restriction of chemical fungicides in the integrated disease management adopted plots, at farmers' field condition, also aided in build-up of *Trichoderma* population in the soil. Similar observations were recorded by Vikram and Hamzehzarghani (2011) while working with sclerotial stem rot management in groundnut.

Conclusion

The present study opened up the scope of using local strains of biocontrol agents and biofumigation potentiality of *Brassica* crops for the eco-friendly management of soil borne pathogens in the coastal saline zone. Biofumigation with Indian mustard offered an economical management of the collar rot disease without compromising the crop yield and the population of *Trichoderma* spp. in soil. Integration of soil solarization, after a proper resting of the soil in the form of a heap, also reduced collar rot disease under field condition.

Abbreviations BCR: Benefit-cost ratio; CFU: Colony forming unit; DI: Disease incidence; FP: Farmer's Practice; IDM: Integrated Disease Management; ₹: Indian Rupee; cv.: Cultivar; syn.: Synonym

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Declarations

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Consent to participate Not required for this study, as the article does not contain any human and animal rights.

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