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Research article

Biorational management of root-knot of brinjal (Solanum melongena L.) caused by Meloidogyne javanica

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ABSTRACT

Production of brinjal (Solanum melongena L.) is considerably reduced by infestations of root-knot nematodes (RKN). As chemical pesticides are increasingly being regulated globally, scientists are focusing on biorational management. An experiment was undertaken to screen resistant brinjal cultivars in Bangladesh against Meloidogne javanica in a pot trial. Pot and field trials were also conducted to evaluate the efficacy and profitability of individual and combined applications of several biorational components to manage M. javanica on brinjal. Of twenty brinjal cultivars screened, cv. Noagram was found 'moderately resistant' and others were 'susceptible' to 'highly susceptible' against M. javanica. In both pot and field trials, most of the growth parameters of brinjal and reproductive parameters of *M. javanica* were significantly different than the control for both the individual and combined treatments of different biorational components which included cabbage, marigold, vermicompost, biogas digestate, Bacillus subtilis and Pseudomonas fluorescens. The yield was significantly higher for the combined treatments than the individual applications. The benefit-cost ratio (BCR) differed among the treatments. The highest yield (29.5 t/ha) and BCR (3.67) with the lowest reproductive factor (0.33) was obtained by the combined application of biogas digestate and B. subtilis. This is the first report on the efficiency and profitability assessment of biogas digestate in combination with a bio-agent in addressing the management of RKN, which might be very important considering the global concern of environmental pollution. The cultivar Noagram might be a potential source of resistant genes in brinjal against M. javanica.

1. Introduction

Brinjal Solanum melongena L. is one of the most popular vegetables in S. Asia, and is consumed worldwide. It is also known as eggplant or aubergine in several countries (Rangarajan et al., 2021). An edible portion of 100 gm brinjal contains 1.4 gm protein, 18 mg calcium, and 24 kcal of food energy (Hasan and Bai, 2016). Production of brinjal is affected by the infestation of several pests which include plant-parasitic nematodes (PPN). Among the PPN, root-knot nematode (RKN) is the most prominent. Globally, three species of RKN, Meloidogyne incognita, M. javanica, and M. arenaria have been found to be associated with the root-knot of brinjal, however, in Bangladesh M. javanica is the most prevalent one (Das et al., 2021c). Globally, 27%-62% yield of brinjal is lost due to the infection of RKN (Darekar and Mhase, 1988; Bari, 2001). Generally, broad-spectrum synthetic chemical pesticides are applied to control RKN. Since these broad-spectrum non-selective pesticides are designed to kill animals, they are also dangerous to human beings, and detrimental to non-target organisms in many ways (Kepenekci et al., 2017). In addition, as a consequence of the long-term use of these chemicals, resistance-breaking nematode pathotypes emerge on many important crops which are guiding policymakers to impose restrictions on various molecules used worldwide (Abu-Elgawad and Askary, 2015). Therefore, as an alternative, the efficacy of biorational components against the infection of RKN is evaluated (Huang et al., 2016).

Plant resistance is the heritable ability of plants to escape the extent of damage caused by pathogens (Stenberg and Muola, 2017). As the most effective and environmentally safe method, plant resistance is the foremost component in integrated pest management. Reasonably, finding resistance in plants against RKN is one of the quests of nematologists. The use of biocontrol agents like fungi and bacteria is of growing interest for controlling PPN non-chemically (Crawford and Clardy, 2011). Two rhizosphere bacteria Bacillus subtilis and Pseudomonas fluorescens have

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been reported to inhibit RKN reproduction, egg hatch, and juvenile survival by producing different enzymes and toxins (Siddiqui and Mahmood, 1999; Das et al., 2021a). As bio-nematicides are formulated based on living systems, there has always been a gap between laboratory and field performance of those (Abu-Elgawad and Askary, 2015).

Bio-fumigation is another non-chemical approach. Here, various plant species are incorporated into the soil to liberate volatiles which suppresses soil-borne pests (Berbegal et al., 2008). For example, marigold and cabbage are important sources of nematicidal compounds (Zasada and Ferris, 2004; Abo-Elyousr et al., 2010; Taye et al., 2012; Youssef and Lashein, 2013). It is already reported that the life cycle and pathogenicity of RKN are adversely affected by marigold (Tagetes spp.) (Abo-Elyousr et al., 2010; Tibugari et al., 2012; Taye et al., 2012). However, its effect on the population of RKN varies depending on the cultivar of marigold and the species of RKN (Karssen and Moens, 2006). It is hypothesized that marigold might exert an antagonistic effect on RKN populations whereby the second-stage juvenile (J₂) fails to initiate giant cells in the roots (Karssen and Moens, 2006). On the other hand, cabbage (Brassica spp.) releases glucosinolates (GSL) which on hydrolysis produce isothiocyanate (ITC) that has a toxic effect on certain nematode species (Zasada and Ferris, 2004). However, incorporation of the appropriate amounts of glucosinolate-containing biomass and sensitivity of the target PPN species to ITC are important determinants of the successful outcome (Zasada and Ferris, 2004).

As an alternative to chemical pesticides, several organic amendments are being investigated to determine their efficacy in controlling PPN, although, results have been inconsistent (Akhtar and Malik, 2000). Vermicompost, composted in the presence of earthworm, is an important organic amendment that increases crop production. Vermicompost results in high porosity, aeration, drainage, water-holding capacities, and low C: N ratios (Edwards, 1998). Vermicompost was reported to decrease RKN-induced galls, and to increase the concentration of root defense metabolites in plants (Xiao et al., 2016). Biogas digestate (BD), produced as a byproduct of a biogas facility, is an organic amendment that improves soil fertility, crop quality, and plant immunity to biotic and abiotic agents (Koszel and Lorencowicz, 2015). To reduce the negative impact of burning fossil fuels, environmentalists and policymakers are considering biodegradable organic waste as an alternative energy source (Koszel and Lorencowicz, 2015). In a biogas facility, organic wastes are anaerobically decomposed in an environment-friendly way to produce biogas (Koszel and Lorencowicz, 2015). It has been reported that BD has some potential to reduce PPN infestation (Min et al., 2007).

Various concentrations of culture filtrates of B. subtilis and P. fluorescens have been reported to strongly influence mortality and hatching of M. javanica (Das et al., 2021a). In an in vitro experiment, the effect of vermicompost, BD, and botanical extracts of marigold and cabbage was evaluated on the hatching and mortality of M. javanica (Das et al., 2021b). Results showed that mortality and inhibition of hatching of M. javanica were higher in botanical extracts than those of organic amendments. However, the soil is a complex ecosystem where results are affected under several physical, chemical, and biological stressors. Moreover, the biocontrol of RKN does not rely on any single measure, but rather an integrated approach (El-Ashry et al., 2021). To be sustainable, control measures need to be economically feasible for the farmers. There are few publications that link effective control measures with an economic return. As M. javanica is the most prevalent RKN of brinjal in Bangladesh, the objectives of this experiment were 1) to screen brinjal cultivars against M. javanica in a pot trial and 2) to evaluate the individual and combined applications of biorational components to manage M. javanica of brinjal under field condition with an assessment of the economic benefit.

2. Materials and methods

2.1. Nematode inoculum

Egg masses of a pure culture of *M. javanica* (NCBI accession no. MN196542) were used in this experiment (Das et al., 2021b).

2.2. Screening of brinjal cultivars for resistance

Twenty brinjal germplasms were considered for screening for resistance. Eight cvs. (BARI begun 1, 4, 5, 6, 7, 8, 9, 10) were improved varieties of Bangladesh Agricultural Research Institute (BARI) and twelve cvs. (Borkha, Black long, Noagram, Shyla, Mental Red, Jhumka, Hari, Hajari begun, Tilokpur, shingnath, Narsingdi, Manikgani) were collected from local farmers of Bangladesh. Seeds were sown into pots at the net house of Seed Pathology Center (SPC), Bangladesh Agricultural University (BAU), Mymensingh under 25-30 °C temperature with 12–13 h of daily daylight. Pots (16 cm \times 25 cm each) were filled with 0.1% formalin-sterilized soil. Each pot received a single seedling of each cultivar with four replications. At thirty days, each seedling was inoculated with two hundred eggs of M. javanica. Sixty days after inoculation, growth parameters of the plant and reproductive parameters of the nematodes were recorded (Begum et al., 2014). Egg masses were stained with acid fuchsin to aid in counting (Bybd et al., 1983). A root-knot index scale was used to resistance, where 1 = no egg mass = highly resistant; 2 = 1-10 eggmass = resistant; 3 = 11-30 egg mass = moderately Susceptible; 4 =31-100 egg mass = Susceptible; 5 = > 100 egg mass = highly susceptible (Gaur et al., 2001). Plants were arranged in a completely randomized design (CRD).

2.3. Preparation and application of treatments

2.3.1. Cabbage and marigold leaves

Cabbage (*B. oleracea*) and marigold (*Tagetes* spp.) leaves were collected from cultivated fields and chopped into 1–2 cm pieces. Chopped cabbage/Marigold leaves were incorporated immediately into the soil @ 20 g/pot (pot trial) and @ 200 g/plot (field trial) of soil 10 days before transplanting of the seedlings (Youssef and Lashein, 2013). Until transplanting, the treated soil was covered with a plastic sheet to retain the volatiles in the soil.

2.3.2. Vermicompost and biogas digestate (BD)

Animal dung and agro/kitchen waste were used to produce vermicompost and biogas (Nath and Singh, 2011). Earthworms (*Eisenia fetida*) were used as a composter @ 2kg/vermibed ($3 \text{ m} \times 1 \text{ m} \times 9\text{m}$). Solid BD was collected from a household biogas facility at Mymensingh, Bangladesh. For the vermicompost-soil mixture and BD-soil mixture treatments, vermicompost and BD were mixed with soil @ 500 g/pot (pot trial) and @ 5 kg/plot (field trial) 10 days before transplanting (Serfoji et al., 2010).

2.3.3. Preparation of B. subtilis and P. fluorescens suspension

Two bacterial strains *B. subtilis* (MN252542.1) and *P. fluorescens* (MN256394.1) were isolated from the rhizosphere soil of Meherpur (24°N, 89°E) and Mymensingh (25°N, 90°E) of Bangladesh, and stored at -80 °C at the Department of Plant Pathology, BAU maintaining proper condition (Schaad, 1980; Krieg and Holt, 1984; Zihad et al., 2021). For bacterial inoculum, *B. subtilis* was grown on nutrient agar and *P. fluorescens* was grown on King's medium B for 24 h at 28 °C (Mahesha et al., 2017). The bacteria were then transferred to nutrient broth in a 250 ml conical flask and incubated for 48 h at 200 rpm at 32 °C (Sela et al., 1998). Bacterial suspensions @ 40 ml/plant (10⁹ CFU/ml) were applied twice, immediately after inoculation (in pot trial)/transplanting (in field trial) and 15 days after the 1st application, in both pot and field trials (Abo-Elyousr et al., 2010).

2.3.4. Chemical pesticide

For a positive control, the organophosphate nematicide, Cadusafos @ 1g/pot (pot trial) and @ 10g/plot (field trial) was mixed into the soil 10 days before transplanting (Abo-Elyousr et al., 2010). Pots or plots with brinjal seedlings receiving no treatments served as a negative control.

Table 1. Treatments of biocomponents applied in the pot and field experiment.

T ₀ = Control	$T_6 =$ Bacterial suspension of Pseudomonas fluorescens
$T_1 = Marigold \ leaf$	$T_7 = Rugby 10G$ (Positive Control)
$T_2 = Cabbage \ leaf$	$T_8=T_3+T_5\\$
$T_3 = Vermicompost$	$T_9=T_3+T_6$
$T_4 = Biogas Digestate$	$T_{10} = T_4 + T_5$
$T_5 = Bacterial suspension of Bacillus subtilis$	$T_{11} = T_4 + T_6$

 $T_8 = T_3$ (Vermicompost @ 500g/pot or 5kg/plot) + T_5 (Bacterial suspension of *Bacillus subtilis* @ 40 ml/plant).

 $T_9 = T_3$ (Vermicompost @ 500g/pot or 5kg/plot) + T_6 (Bacterial suspension of *Pseudomonas fluorescens* @ 40 ml/plant).

 $T_{10}\,{=}\,T_4$ (Biogas Digestate @ 500g/pot or 5kg/plot) + T_5 (Bacterial suspension of Bacillus subtilis @ 40 ml/plant).

 $T_{11} = T_4$ (Biogas Digestate @ 500g/pot or 5kg/plot) + T_6 (Bacterial suspension of *Pseudomonas fluorescens* @ 40 ml/plant).

2.4. Evaluation of different biorational components against RKN (pot trial)

Pots were prepared the same as for resistance screening. The susceptible cv. BARI begun 4 (Begum et al., 2014) was used for evaluating the biorational components. Each pot received a single seedling with 4 replications per treatment (Table 1). After thirty days, each seedling was inoculated with two hundred eggs of *M. javanica*. Data on growth parameters of plants and reproductive parameters of nematodes were recorded 45 days after inoculation (DAI) and 90 DAI (Akhter and Khan, 2018). Eggs were extracted from egg masses following the sugar floatation method with 40% sucrose solution (Hussey and Barker, 1973), and J₂ from soil was extracted by a modified centrifugal-flotation technique (Jenkins, 1964). Egg masses on the infected roots were counted by staining with acid fuchsin (Bybd et al., 1983). Plants were arranged in a CRD.

Table 2. Growth responses and resistance (root-knot index) of brinial cultivars infected by Meloidogyne javanica.

2.5. Evaluation of different biorational components against RKN (field trial)

The field experiment was conducted at a farmer's field naturally infested with M. javanica (Das et al., 2021c). The field was located at Madhupur (24.61°N, 90.03°E) of the Tangail district where brinjal is cultivated year-round (Das et al., 2021c). To assess the level of infestation, 10 soil samples were collected 15 cm deep in each of the plots. The plots were 3.5 square meters. The number of nematodes was expressed as the number of J₂/100g soil. The field plots had sandy loam soil with a pH of 6.4. A humid tropical climate prevailed with an average temperature of 25-34 °C during the experiment period. The plots were prepared by standard agronomic practices. In each plot, six thirty-day-old cv. BARI begun 4 were transplanted with a spacing of 75 \times 50 cm and standard levels of fertilizers (SRDI, 2021), irrigation, and pesticides (when necessary) were provided. Each plot was applied with any one of the treatments and was replicated three times. Plots were arranged in an RCBD. Data on plant growth and yield and reproductive parameters of M. javanica were recorded 90 days after transplanting (DAT). Data from each of the six plants per plot were averaged. Staining of egg masses, and the extraction of J_2 and eggs from soil and egg masses, respectively, were conducted as for the pot trial. The cost of cultivation of brinjal was estimated by the method of Hasan and Bai (2016). The benefit-cost ratio (BCR) was calculated using the formula:

 $BCR = (A \times C) \div B$ (Reddy and Reddy, 1992).....(1) Where, A = Yield (kg/ha), B = Cost of cultivation of the crop (Tk/ha), C = Price of the product (Tk/kg), Tk = Bangladeshi Taka/Currency.

2.6. Statistical analysis

Data were subjected to an analysis of variance (ANOVA) using Statistix 10 (© 1985–2013 Analytical Software, Miller Landing Rd, Tallahassee, FL 32312) and significant means were separated using Tukey's HSD test at 5% level of probability. For resistance screening, a pairwise T-

Cultivar	Shoot length (cm)		Root length (cm)		Shoot w (g)	Shoot weight (g)	Root weight (g)		Leaf number		Branch number		Root-knot Index	
	С	I	С	I	С	I	С	I	С	I	С	I	С	I
BARI begun 1	77.5	71.5*	11.5	14.25*	75.5	56.5*	23.25	18.25*	20.5	17.5	4	3	0	5
BARI begun 4	57	50*	21.75	18.75*	79.5	58.5*	25.5	17.5*	20.75	18.75	4.25	3.5	0	5
BARI begun 5	62.5	50*	17	12.25*	65.25	41.75*	18.75	12.5*	16	14.75	4	3.5	0	4
BARI begun 6	76.75	66.5*	25.25	18.5*	86.75	67.25*	23	17.75*	18.5	16	4.5	3.75	0	5
BARI begun 7	64.75	53.5*	25	21.5*	87.5	71*	25.5	16.75*	14.5	12	3	3.5	0	5
BARI begun 8	75.75	61.25*	20.75	14.5*	82	55.25*	23	12.75*	16.5	13.75	3.75	2.75	0	5
BARI begun 9	68.5	50.25*	23.75	18.25*	58.75	46*	21	11.5*	14.25	12.75	3.75	3	0	5
BARI begun 10	74	59.75*	20.75	13.5*	90	33.5*	22	16*	11.75	16	3.25	2.5	0	5
Borkha	65.25	51.75*	28.25	21.25*	46.75	29.25*	25.25	17.5*	14.25	13.5	2.75	2.5	0	5
Noagram	54.75	52.25	56.25	53.5	67.75	65.25	16.5	14.25	14.5	12.75	2.5	2.5	0	3
Black long (Black long)	61.75	52.5*	38.0	29.25*	51.75	41.75*	14.5	10.75*	14	12.5	2	2	0	4
Shyla	64.25	53*	58.25	44.25*	69	58.25*	28.5	22.75*	15	13.75	2.75	2.5	0	5
Mental Red	60.75	49.25*	44.75	34.75*	46.75	38.75*	15.25	12*	17.25	16	3	2.25	0	5
Jhumka	65	55*	26.75	21.25*	48.5	38.5*	20.25	12.75*	26	22.75	5.25	4.5	0	4
Hari	57.5	48.25*	28	21.25*	48	40.5*	15	10.5*	14.75	12.75	3	2.5	0	5
Hajari begun	64	54.75*	43.75	33*	61.25	49*	21.75	14.5*	19.5	17	3	2.5	0	4
Tilokpur	63.5	52*	29.5	23*	48.5	40.75*	17.5	11.25*	16	12.75	2.75	2.25	0	5
Shingnath	59.75	49.75*	29.75	22.25*	62.5	55*	16.75	12.5*	14.25	12.25	2.5	2	0	4
Narsingdi	65	53.75*	27.75	21.75*	59.75	41*	20.25	13*	13	12	2.25	2	0	5
Manikganj shingnath	59.5	48.5*	31	23.5*	46.5	35*	24.75	15.25*	11.25	10	2	2	0	5

Means are the average of four replications; C = Control; I = inoculated; * = Significantly different in pairwise T test; Root-knot Index = 1 = no egg mass = highly resistant; 2 = 1-10 egg mass = resistant; 3 = 11-30 egg mass = moderately resistant; 4 = 31-100 egg mass = Susceptible; 5 = >100 egg mass = highly susceptible (Gaur et al., 2001).

Table 3. Effects of biocomponents on the reproductive parameters of *Meloidogyne javanica* at 45 DAI in a susceptible brinjal cultivar (BARI begun 4) in pots.

Treatment	Root (3g)		$J_2/100g$ soil	
	Gall	Egg mass	Egg	
T ₀	$25\pm1.78a$	$19\pm1.41a$	$285\pm21.21a$	$200\pm14.85a$
T1	$5.5\pm1.04def$	$0\pm0e$	$0\pm0e$	$0\pm 0e$
T ₂	$10.25\pm1.11 \text{cd}$	$5.5\pm0.65cd$	$66 \pm 4.33 cd$	$36.25 \pm 4.33 cd$
T ₃	$15.75\pm0.95b$	$11\pm0.71b$	$165\pm10.61b$	$99\pm 6.36b$
T ₄	$13\pm1.29 bc$	$\textbf{7.75} \pm \textbf{0.85c}$	$100.75\pm11.1c$	$57.25\pm 6.37c$
T ₅	$4\pm0.91 f$	$0\pm 0e$	$0\pm0e$	$0\pm 0e$
T ₆	$4.5\pm0.87\text{ef}$	$0\pm0e$	$0\pm0e$	$0\pm 0e$
T ₇	$3.25\pm0.25 f$	$0\pm0e$	$0\pm0e$	$0\pm 0e$
T ₈	$\textbf{9.25} \pm \textbf{0.48cde}$	$\textbf{4.75} \pm \textbf{0.25d}$	$57\pm 3d$	$25.75 \pm 1.25 \text{de}$
Т9	$\textbf{9.75} \pm \textbf{1.11cd}$	$5.25\pm0.48cd$	$63\pm5.74cd$	$28.25\pm2.39d$
T ₁₀	$8\pm0.41\text{def}$	$3.75\pm0.25d$	$45\pm 3d$	$20.5 \pm 1.5 \text{de}$
T ₁₁	$\textbf{9.25}\pm\textbf{0.85cde}$	$\textbf{4.25} \pm \textbf{0.48d}$	$51\pm5.74d$	$23\pm2.61\text{de}$
CV (%)	20.46	23.26	23.96	25.94
F-value	36.59	88.2	101.25	119.23
df	11			
Level of signi	ficance *			

Values are the mean \pm Standard Error of four replicates; Treatment means were compared by one-way ANOVA; Same letter in a column do not differ significantly according to Tukey's test at 5% probability; DAI = Days after incubation *1% level of probability; T₀ = Control, T₁ = Marigold leaf, T₂ = Cabbage leaf, T₃ = Vermicompost, T₄ = Biogas Digestate,T₅ = Bacterial suspension of *Bacillus subtilis*, T₆ = Bacterial suspension of *Pseudomonas fluorescens*, T₇ = Rugby 10G (Positive Control), T₈ = T₃ + T₅, T₉ = T₃ + T₆, T₁₀ = T₄ + T₅, T₁₁ = T₄ + T₆.

test was performed using MS Excel to differentiate the parameters between control and inoculated plants.

3. Results

3.1. Screening of brinjal cultivars for resistance to RKN

All of the BARI varieties were 'susceptible' to 'highly susceptible' to RKN, with a root-knot index of 4–5 (Table 2). All of the local cultivars

except 'Noagram', had a root-knot index 4 to 5. The cultivar 'Noagram' had a root-knot index of 3. Growth parameters of the germplasms were also studied. The number of branches and leaves was not significantly different between the control and inoculated plants in any of the cultivars. The rest of the growth parameters (shoot length, root length, shoot weight, root weight) were found significantly different than that of the control in the pairwise T-test for all cultivars except 'Noagram'. There was no significant difference between the control and the inoculated plants in any growth parameters for the cultivar 'Noagram', suggesting that this cultivar has moderate resistance against the infestation of *M. javanica*.

3.2. Evaluation of biorational components against RKN (pot trial)

All of the reproductive parameters of *M. javanica* differed significantly at 45 and 90 DAI (Tables 3 and 4). AT 45 DAI, the lowest number of galls were found in the treatment T_5 and T_7 , while no egg masses, eggs, and J_2 were observed for the treatment T_1 , T_5 , T_6 , and T_7 . At 45 DAI, the secondhighest number of galls, egg masses, eggs, and J_2 were observed for the treatment T_3 . At 90 DAI, the lowest number of galls were recorded in the treatment T_7 , however, a statistically similar number of galls were found in some other treatments (T_1 , T_5 , T_6 , T_8). Similarly, the lowest number of egg masses were found in the treatment T_7 , and some other treatments (T_1 , T_2 , T_5 , T_8) had statistically similar counts for the same variable. The number of eggs was the lowest for the treatment T_1 and T_5 , and the number of extracted J_2 from soil was the lowest in T_1 , T_5 , T_6 , and T_7 at 90 DAI. All of the reproductive parameters had the highest count in T_0 at both 45 and 90 DAI.

In the pot experiment, all the growth parameters of plants, except branch numbers, differed significantly at both 45 and 90 DAI (Tables 5 and 6). Root length was the highest in T_5 and T_7 , while the maximum leaf number was observed in T_5 at 45 DAI. At 90 DAI, shoot length, root length, shoot weight, and root weight was the highest in the treatment T_{10} . At both 45 and 90 DAI, all of the growth parameters of plants were the lowest in T_0 .

3.3. Evaluation of biorational components against RKN (field trial)

All of the growth parameters of plants, *viz.* shoot length (F-value = 24.73, df = 11), root length (F-value = 11.44, df = 11), shoot weight

Table 4. Effects of biocomponents of	n the reproductive paramet	ers of Meloidogyne javanica at 90 I	DAI in a susceptible brinjal cultivar	(BARI begun 4) in pots.

Treatment	Root (3g)			J ₂ /100g soil
	Gall	Egg mass	Egg	
T ₀	$140\pm5.96a$	$82.75 \pm 1.93a$	$1820.5\pm42.48a$	1459 ± 33.65a
T ₁	17 ± 1.87 cd	13 ± 1.68 cde	$125\pm11.52 \mathrm{f}$	$75\pm 6.98e$
T ₂	$30.5\pm2.1c$	$22.5\pm2.18 cde$	$337.5\pm32.69d$	$239 \pm 21.52 d$
T ₃	$89\pm4.55b$	$49.5\pm3.77b$	$891\pm67.95b$	$705.5\pm54.36b$
T ₄	$82\pm5b$	$43.75\pm3.04b$	$656.25\pm45.57c$	$\textbf{457.75} \pm \textbf{29.6c}$
T ₅	$16.75\pm1.75cd$	$12.75\pm2.38 de$	$123.75\pm10.13 \mathrm{f}$	$74.5 \pm 6.22 e$
T ₆	$17.5 \pm 1.76 cd$	14 ± 1.68 cde	$136.75\pm5.88ef$	$82\pm3.49\text{e}$
T ₇	13.75 ± 1.11d	$11.5\pm1.19e$	$128\pm 6.86 f$	$\textbf{76.75} \pm \textbf{4.17e}$
T ₈	$26.25\pm1.89 cd$	$20.5\pm2.99 cde$	307.5 ± 44.79 de	$215.25 \pm 31.35d$
T9	$30.5\pm2.22c$	$24\pm2.08c$	$360 \pm \mathbf{31.22d}$	$254.25 \pm 22.48d$
T ₁₀	$29\pm0.91c$	$23.5\pm1.71 cd$	$352.5\pm25.62d$	$230.5\pm16.3d$
T ₁₁	$29.5 \pm 1.94 c$	$23.75\pm2.06cd$	$356.25\pm30.85d$	$244\pm21.89d$
CV (%)	13.90	15.89	14.90	14.85
F-value	168.19	84.88	194.87	242.72
df	11			
Level of significance *				

Level of significance ^

Values are the mean \pm Standard Error of four replicates; Treatment means were compared by one-way ANOVA; Same letter in a column do not differ significantly according to Tukey's test at 5% probability; DAI = Days after incubation *1% level of probability; T₀ = Control, T₁ = Marigold leaf, T₂ = Cabbage leaf, T₃ = Vermicompost, T₄ = Biogas Digestate, T₅ = Bacterial suspension of *Bacillus subtilis*, T₆ = Bacterial suspension of *Pseudomonas fluorescens*, T₇ = Rugby 10G (Positive Control), T₈ = T₃ + T₅, T₉ = T₃ + T₆, T₁₀ = T₄ + T₅, T₁₁ = T₄ + T₆.

Table 5. Effects of biocomponents on the growth parameters of a susceptible brinjal cultivar (BARI begun 4) in response to inoculation with Meloidogyne javanica at 45
DAI in pots.

Treatment	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	Branch number	Leaf number
T ₀	34.5 ± 2.39b	16.75 ± 1.03b	21.75 ± 2.69b	11.25 ± 0.85b	1.5 ± 0.29	$8.5\pm0.65b$
T ₁	$51 \pm 1.35a$	$26 \pm 1.29 ab$	$42\pm2.12a$	$21\pm0.91a$	1.75 ± 0.25	$11.25\pm0.63 \mathrm{ab}$
T2	$52.75\pm4.59a$	$\textbf{25.75} \pm \textbf{2.5ab}$	$43.25 \pm \mathbf{3.42a}$	$\textbf{20.5} \pm \textbf{1.19a}$	1.5 ± 0.29	$11\pm0.71 ab$
T ₃	$53.75 \pm 2.06 a$	$25.75\pm2.39ab$	$44.5 \pm \mathbf{1.85a}$	$\textbf{20.25} \pm \textbf{1.11a}$	1.25 ± 0.25	$10.5\pm0.87 ab$
T ₄	$51\pm0.71a$	$25\pm2.68 ab$	$42.75\pm2.5a$	$19\pm1.08 \text{a}$	1.5 ± 0.29	$11.25\pm0.48 \text{ab}$
T ₅	$51.25\pm2.56a$	$27 \pm \mathbf{2.12a}$	$46.5 \pm \mathbf{2.53a}$	$21.25 \pm \mathbf{1.25a}$	1.75 ± 0.25	$12\pm0.41\text{a}$
T ₆	$\textbf{50.75} \pm \textbf{1.44a}$	$23.5\pm2.22 ab$	$44.75 \pm \mathbf{2.28a}$	$19\pm1.41a$	1.75 ± 0.25	$11\pm0.41 ab$
T ₇	$\textbf{50.75} \pm \textbf{1.43a}$	$\textbf{27.5} \pm \textbf{1.04a}$	$44.5\pm2.25a$	$19.75\pm0.85a$	1.5 ± 0.29	$11.75\pm0.75 ab$
T ₈	$55.5 \pm \mathbf{5.24a}$	$26\pm1.58 ab$	$43\pm2.04a$	$22 \pm \mathbf{1.22a}$	2 ± 0	$11\pm0.41 ab$
Т9	$56.25 \pm \mathbf{3.5a}$	$26.25\pm2.75ab$	$43.5\pm3.5a$	$19.75\pm0.85a$	1.75 ± 0.25	$11.5\pm1.19 ab$
T ₁₀	$57.25\pm2.78a$	$25.75\pm0.75ab$	$46 \pm \mathbf{1.87a}$	$21.5\pm1.55a$	2 ± 0	$12.25\pm0.75b$
T ₁₁	$53.5\pm3.61a$	$24.5\pm1.94\text{ab}$	$44\pm3.03a$	$21.25 \pm \mathbf{1.88a}$	1.5 ± 0.29	$10.25\pm0.48ab$
CV (%)	11.46	15.8	12.15	12.39	29.95	12.36
F-value	3.89	2.01	6.57	5.39	-	2.07
df	11					
Level of cignifica	nco *					

Level of significance *

Values are the mean \pm Standard Error of four replicates; Treatment means were compared by one-way ANOVA; Same letter in a column do not differ significantly according to Tukey's test at 5% probability; DAI = Days after incubation *1% level of probability; T₀ = Control, T₁ = Marigold leaf, T₂ = Cabbage leaf, T₃ = Vermicompost, T₄ = Biogas Digestate, T₅ = Bacterial suspension of *Bacillus subtilis*, T₆ = Bacterial suspension of *Pseudomonas fluorescens*, T₇ = Rugby 10G (Positive Control), T₈ = T₃ + T₅, T₉ = T₃ + T₆, T₁₀ = T₄ + T₅, T₁₁ = T₄ + T₆.

(F-value = 31.58, df = 11), root weight (F-value = 10.85, df = 11), branch number (F-value = 4.13, df = 11), leaf number (F-value = 10.07, df = 11) and yield (F-value = 96.26, df = 11) differed significantly (Table 7). In the field trial, shoot length was the highest in T_7 and T_{10} . However, for other growth parameters, the treatment T_{10} was superior. The highest and statistically similar yield of brinjal was recorded in all of the combined treatments, i.e., T_8 , T_9 , T_{10} , and T_{11} . R_f was the lowest (0.33) in T_{10} . On the other hand, significantly the lowest growth parameters of plants and yield were recorded in T_0 , while the R_f was the highest (2.68) in this treatment. The efficacy of all of the treatments was also analyzed in the light of BCR (see Eq. (1)) to determine their economic feasibility (Table 8). As higher yield was obtained in the combined treatments, higher revenues were also obtained from those treatments (T₈, T₉, T₁₀, and T₁₁). The highest BCR (3.67) was recorded for the treatment T₁₀ which was followed by T₁₁ (3.4). Although total revenue was almost similar to T₁₀ and T₁₁, the BCR was much lower (2.59) in the treatment T₈ and T₉. On the contrary, BCR for the treatment T₅ (3.23), T₆ (3.23), and T₇ (3.26) were much closer to the highest one despite having much lower revenue than that of T₁₀ and T₁₁.

Table 6. Effects of biocomponents on the growth parameters of a susceptible brinjal cultivar (BARI begun 4) in response to inoculation with *Meloidogyne javanica* at 90 DAI in pots.

Treatment	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	Branch number	Leaf number
To	$38.5 \pm \mathbf{1.94e}$	$18.75\pm1.31\mathrm{e}$	$22.75\pm1.1\mathrm{d}$	$15.75 \pm 1.5 \mathrm{c}$	1.75 ± 0.25	$11.75\pm0.85b$
T1	$53.25\pm2.17 cd$	$26.75\pm0.75cd$	$46 \pm 1.08 abc$	$\textbf{22.75} \pm \textbf{1.44ab}$	2.25 ± 0.25	$14.25\pm1.11 \mathrm{ab}$
T ₂	$53\pm3.03cd$	$26.25\pm1.31\text{d}$	$44.5 \pm 1.85 bc$	$22\pm1.08bc$	2.25 ± 0.25	$14\pm0.71b$
T ₃	$54.25\pm1.89~\text{(a-d)}$	$26\pm1.58d$	$45\pm2.04bc$	$21.5\pm1.04bc$	2.5 ± 0.29	$16\pm1.08ab$
T ₄	$52\pm2.12d$	$25.75 \pm 1.31 d$	$43\pm1.59c$	$22.5\pm1.32ab$	2.25 ± 0.25	$14.25\pm1.18 ab$
T ₅	$53.5\pm2.25 bcd$	$\textbf{27.5} \pm \textbf{1.19bcd}$	$47.25\pm2.78abc$	$23.5\pm1.25 ab$	$\textbf{2.75} \pm \textbf{0.25}$	$15.75\pm1.49 ab$
T ₆	$51.5\pm1.55 d$	$26\pm1.47d$	$45.5\pm1.32 bc$	$23.25\pm1.25 ab$	$\textbf{2.25} \pm \textbf{0.25}$	$15\pm0.82ab$
T ₇	$51.25 \pm 1.31 d$	28.5 ± 0.65 (a-d)	$45.75\pm0.85bc$	$24\pm1.22 ab$	3 ± 0.41	$14.5\pm1.55ab$
T ₈	$62.25\pm0.48ab$	$\textbf{32.25} \pm \textbf{0.63ab}$	$51.5\pm1.71 ab$	$\textbf{27.25} \pm \textbf{1.65ab}$	3 ± 0	$16.5\pm0.65 ab$
Т9	$61.75 \pm 1.18 abc$	$32\pm0.91 abc$	$51.25 \pm 1.8 ab$	$27\pm1.22 ab$	3 ± 0.41	$15\pm1.47 ab$
T ₁₀	$62.5 \pm \mathbf{0.86a}$	$33.25 \pm \mathbf{0.63a}$	$53.75 \pm 1.03 a$	$29\pm1.83a$	3.25 ± 0.48	$19.5\pm0.65a$
T ₁₁	$61\pm0.82abc$	$32.25\pm0.85ab$	$51.75\pm0.95ab$	$28\pm1.47 ab$	3 ± 0.41	$15.5\pm1.04ab$
CV (%)	6.52	7.9	7.02	11.21	24.16	14.45
F-value	14.52	13.7	24.95	7.27	-	2.79
df	11					
Level of significa	ance *					

Values are the mean \pm Standard Error of four replicates; Treatment means were compared by one-way ANOVA; Same letter in a column do not differ significantly according to Tukey's test at 5% probability; DAI = Days after incubation *1% level of probability; T₀ = Control, T₁ = Marigold leaf, T₂ = Cabbage leaf, T₃ = Vermicompost, T₄ = Biogas Digestate, T₅ = Bacterial suspension of *Bacillus subtilis*, T₆ = Bacterial suspension of *Pseudomonas fluorescens*, T₇ = Rugby 10G (Positive Control), T₈ = T₃ + T₅, T₉ = T₃ + T₆, T₁₀ = T₄ + T₅, T₁₁ = T₄ + T₆.

Table 7. Effect	ts of biocomponents on 1	the growth parameters ar	nd yield of a susceptible	brinjal brinjal cultivar (]	BARI begun 4), and rej	Table 7. Effects of biocomponents on the growth parameters and yield of a susceptible brinjal brinjal cultivar (BARI begun 4), and reproductive factor (R _f) of <i>Meloidogyne javanica</i> at 90 DAT in a farmer's field.	feloidogyne javanica at	t 90 DAT in	a farmer's field.	
Treatment	Shoot length	Root length	Shoot weight	Root weight	Branch number	leaf number	Yield	Pi	Pf	R _f
	(cm)	(cm)	(g)	(g)			(ton/ha)	J ₂ /100g soil	oil	
T_0	87 ± 4.62e	$18.33 \pm 1.76f$	609 ± 32.33e	$40.33\pm3.84\mathrm{e}$	$6.67\pm\mathbf{0.33c}$	77.33 ± 4.33d	$15.81\pm0.13c$	240	642	2.68
T_1	$104 \pm 4.58 de$	26.33 ± 0.88 (b-e)	$761 \pm 14.64 \mathrm{cd}$	$58 \pm 2.08 bcd$	$11.33\pm0.88abc$	$147.33 \pm 11.46 \mathrm{bc}$	$17.71\pm0.33 \mathrm{bc}$	240	168.33	0.7
T_2	$106.67\pm3.92 \mathrm{cde}$	$25 \pm 0.58 de$	$746.67\pm27.51cd$	$55 \pm 1.15cd$	$9.33 \pm \mathbf{0.88bc}$	121.33 ± 11.46 cd	$17.52\pm0.25bc$	240	365	1.52
T_3	94 ± 3.79de	$24.67 \pm 1.2 \mathrm{ef}$	$658\pm\mathbf{26.5de}$	$54.67\pm2.73 de$	$11.67\pm0.88 abc$	$163.33\pm12.34\mathrm{abc}$	$17.9\pm0.415bc$	240	516.67	2.15
T_4	105.33 ± 2.6 cde	25.33 ± 1.76 cde	$737.33 \pm 18.22cd$	$55.67 \pm 4.09cd$	$10 \pm 1.52 bc$	$140\pm21.38\mathrm{bc}$	$18.76\pm0.41b$	240	516.67	2.15
T_5	$111.67 \pm 4.05 bcd$	28.33 ± 1.2 (a-e)	$781.67 \pm \mathbf{28.38c}$	62.33 ± 2.73 (a-d)	$10.33 \pm 1.2 \mathrm{abc}$	$134.33\pm15.62bcd$	$18.29 \pm \mathbf{0.66bc}$	240	136.33	0.57
T_6	108 ± 3.78 cde	$26.33 \pm 2.02 \text{ (b-e)}$	$756\pm26.5cd$	$58 \pm 4.35 bcd$	$9.67\pm0.33\mathrm{bc}$	$122\pm6.08cd$	$18.29\pm0.43 \mathrm{bc}$	240	173.33	0.72
T_7	$151.33 \pm 4.09a$	28.67 ± 0.88 (a-e)	$847.33\pm15.8bc$	$63 \pm 2.08 \; (a-d)$	$11.67\pm1.86 abc$	$150.67\pm10.36\mathrm{bc}$	$18.57\pm0.19b$	240	160	0.67
T ₈	$132.67\pm6.74ab$	31.67 ± 0.33 abc	$932.67\pm12.77 ab$	$69.33\pm0.88abc$	$11.33\pm0.88 abc$	$180.33\pm3.53ab$	$27 \pm 0.76a$	240	108.33	0.45
T ₉	$126.33\pm3.52 \mathrm{bc}$	31.33 ± 0.88 (a-d)	937 ± 17.78ab	69 ± 2.08 (a-d)	$12 \pm 1.53 abc$	184.33 ± 8.41ab	$27\pm0.76a$	240	113.33	0.47
T ₁₀	$153.33 \pm 3.18a$	$33.33 \pm 0.88a$	$1049.33 \pm 18.66a$	$73.33\pm2.02a$	$16\pm0.57a$	$210.33 \pm 6.64a$	$29.5\pm0.58a$	240	80	0.33
T_{11}	$131.67 \pm 4.05ab$	$32.33 \pm 1.2ab$	$937.67\pm21.68ab$	$71.33\pm2.73ab$	$13\pm0.58ab$	$177.33\pm6.06abc$	$27.33\pm0.44a$	240	115	0.48
CV (%)	6.31	7.84	4.92	8.02	17.33	12.87	4.13			
Level of significance *	ance *									
# Values are th after transplam Pseudomonas fl	he mean \pm Standard Erro tation *1% level of prob <i>uorescens</i> , T ₇ = Rugby 10	# Values are the mean \pm Standard Error of three replicates; Treatment means w after transplantation *1% level of probability; $T_0 = Control$, $T_1 = Marigold$ le <i>Pseudomonas fluorescens</i> , $T_7 = Rugby 10G$ (Positive Control), $T_8 = T_3 + T_5$, T_9	tment means were compi = Marigold leaf, $T_2 = C$ = $T_3 + T_5$, $T_9 = T_3 + T_6$	tred by one-way ANOVA abbage leaf, $T_3 = Vermi$, $T_{10} = T_4 + T_5$, $T_{11} = T_4$; Same letter in a colum icompost, T ₄ = Biogas 4 + T ₆ ; Pi = Initial pop	# Values are the mean \pm Standard Error of three replicates; Treatment means were compared by one-way ANOVA; Same letter in a column do not differ significantly according to Tukey's test at 5% probability; DAT = Days after transplantation *1% level of probability; T ₀ = Control, T ₁ = Marigold leaf, T ₂ = Cabbage leaf, T ₃ = Vermicompost, T ₄ = Biogas Digestate, T ₅ = Bacterial suspension of <i>Bacillus subtilis</i> , T ₆ = Bacterial suspension of <i>Pseudomonas fluorescens</i> , T ₇ = Rugby 10G (Positive Control), T ₈ = T ₃ , T ₁₆ = T ₄ , T ₅ , T ₁₁ = T ₄ + T ₅ , T ₁₁ = T ₄ + T ₅ , Pi = Initial population; Pf = Final population; Rf = Reproductive factor (Pf/Pi), ha = hectare, J ₂ = Pseudomonas fluorescens, T ₇ = Rugby 10G (Positive Control), T ₈ = T ₃ , T ₁₆ = T ₄ , T ₅ , T ₁₁ = T ₄ + T ₅ , Pi = Initial population; Pf = Final population; Rf = Reproductive factor (Pf/Pi), ha = hectare, J ₂ = Pseudomonas fluorescens, T ₇ = Rugby 10G (Positive Control), T ₈ = T ₅ , T ₁₀ = T ₄ , T ₅ , T ₁₁ = T ₄ + T ₅ , Pi = Initial population; Pf = Final population; Rf = Reproductive factor (Pf/Pi), ha = hectare, J ₂ = Pseudomonas fluorescens, T ₇ = Rugby 10G (Positive Control), T ₈ = T ₅ , T ₁₀ = T ₄ , T ₅ , T ₁₁ = T ₄ + T ₅ , Pi = Initial population; Pf = Final population; Rf = Reproductive factor (Pf/Pi), ha = hectare, J ₂ = Pseudomonas fluorescens, T ₇ = Rugby 10G (Positive Control), T ₈ = T ₅ , T ₁₀ = T ₄ , T ₅ , T ₁₁ = T ₄ + T ₅ , Pi = Initial population; Pf = Final population; Rf = Reproductive factor (Pf/Pi), ha = hectare, J ₂ = Pseudomonas fluorescens, T ₇ = Rugby 10G (Positive Control), T ₈ = T ₅ , T ₁₀ = T ₄ , T ₅ , Pi = Rugby Pseudomonas fluorescens, Pseudomonas fluorescens, T ₇ = Rugby 10G (Positive Control), T ₈ = T ₅ , T ₁₀ = T ₄ , T ₅ , T ₁₀ = T ₄ + T ₅ , Pi = Rugby Pseudomonas fluorescens, T ₇ = Rugby 10G (Pseudomonas fluorescens) = Rugby Pseudomonas fluorescens, T ₇ = Rugby Pseudomonas fluorescens) = Rugby Pseudomonas fluorescens T ₇ = Rugby Pseudomonas fluorescens, T ₇	ly according to Tukey' uspension of <i>Bacillus</i> : tion; Rf = Reproducti	's test at 5% subtilis, T ₆ = ve factor (P	 probability; DAT - = Bacterial suspens f/Pi), ha = hectar. 	= Days iion of 3, J_2 =

4. Discussion

Brinjal is susceptible to the infection of several species of RKN (Anwar and Mckenry, 2010). Cultivation of resistant cultivars is one of the most desirable components of integrated pest management (Barzman et al., 2015). In this experiment, twenty brinjal cultivars were evaluated for their responses to infection by *M. javanica*. Of the twenty cultivars, only 'Noagram' was found to be 'moderately resistant', and the responses of the rest of the cultivars were 'susceptible' to 'highly susceptible'. These findings are supported by two similar screening experiments conducted in Bangladesh and India, where very few brinjal cultivars were found to have significant resistance to RKN (Begum et al., 2014; Akhter and Khan, 2018). In most screening trials, RKN is considered a generalized pest, although the combination of crop and pest species plays an important role in the outcome of resistance or susceptibility. In a moderately resistant plant, nematodes fail to produce enough functional feeding sites in the host leading to the failure of developing reproducing females (Williamson and Kumar, 2006). For decades, Solanum peruvianum derived Mi gene has been the only source for conferring resistance to M. incognita, M. javanica, and M. arenaria in all commercial tomato cultivars (Seid et al., 2015). Some other resistance genes have also been identified in other crops that are effective against RKN e.g., Me and N genes from pepper, and Mj gene in carrot (Ali et al., 2014). However, there have been no reports of a resistance gene in brinjal. Therefore, the findings of this experiment should be useful for further research on resistance screening against M. javanica in brinjal plants as well as other solanaceous crops.

In the pot trial, the effect of the individual applications of crushed marigold leaves (T1) on egg masses, eggs, and J2's in soil was statistically similar to the positive control (T₇). Similarly, the R_f of the nematode population (0.7) for this treatment was close to the positive control (0.67) in the field trial. Ploeg (2000) also had similar findings as the application of marigold cultivars gave statistically similar results with methyl iodide fumigation for controlling RKN. Marigold releases alpha-terthienyl, an oxidative stress-inducing chemical, which has nematicidal, insecticidal, antiviral, and cytotoxic properties (Hamaguchi et al., 2019). Although no egg masses, eggs, and J₂'s were observed for this treatment (T_1) at 45 DAI in the pot trial, they were present at 90 DAI. It is assumed that over the course of time the influence of chopped marigold leaves on the reproduction of nematodes was reduced as the treatment was applied ten days before transplanting. It was reported that stem extracts were less efficacious than whole T. erecta plant extracts and alpha-terthienyl is only released by active, living marigold roots and becomes inactivated on exposure to near-UV light (Ploeg, 2000; Natarajan et al., 2006). Moreover, not all marigold cultivars control all types of nematodes (Krueger et al., 2019). The findings of this experiment showed that the studied growth parameters and yield were not much influenced by the marigold treatment in comparison to the control. Likewise, comparatively lower BCR (2.99) also would rule out marigold as a profitable management option against the RKN infection. In terms of the studied growth, yield, and reproductive parameters, the individual application of crushed cabbage leaves (T₂) produced significantly better results than the control but inferior to some other treatments (T_1 , T_5 , T_6 , T₇) in both pot and field trials. The reason for less efficacy may be explained by the fact that GSL profiles vary among plant species and cultivars, and their ITC derivatives differ in toxicity to nematodes (Zasada and Ferris, 2004). Moreover, the effect of cabbage on PPN could be inconsistent because the enzymatic conversion of GSL to ITC is temperature-dependent (Lazzeri et al., 2009). In a similar experiment, Youssef and Lashein (2013) did not find an increase in plant growth parameters due to the immediate phytotoxicity exerted by the brassica. They suggested that quick decomposition, rapid evaporation and partial loss of volatile compounds after removing the plastic sheet during the transplanting process and before nematode inoculation could limit the efficacy of Brassica crops in controlling PPN. In the field trial, comparatively lower BCR (2.96) was estimated for this treatment (T₂) which

Juveniles.

Table 8. Benefit-Cost Ratio (BCR) of brin	ial cultivation with the application of b	iocomponents to manage Meloidogyne	javanica in a farmer's field.

Treatment	Seed cost (Tk/ha)	Plowing Cost (Tk/ha)	Labour Cost (Tk/ha)	Fertilizer Cost (Tk/ha)	Irrigation Cost (Tk/ha)	Treatment Cost (Tk/ha)	Total Cost (Tk/ha)	%Yield increase Over control	Total Revenue (Tk/ha)	*BCR
T ₀	18,000	24,000	1,00,000	10,000	14,000	-	1,66,000	-	4,74,300	2.85
T ₁	18,000	24,000	1,00,000	10,000	14,000	11,428	1,77,428	12	5,31,300	2.99
T ₂	18,000	24,000	1,00,000	10,000	14,000	11,428	1,77,428	10.81	5,25,600	2.96
T ₃	18,000	24,000	1,00,000	10,000	14,000	1.42,850	3,08,850	13.2	5,37,000	1.73
T ₄	18,000	24,000	1,00,000	10,000	14,000	71,425	2,37,425	18.65	5,62,800	2.37
T ₅	18,000	24,000	1,00,000	10,000	14,000	3,500	1,69,500	15.62	5,48,700	3.23
T ₆	18,000	24,000	1,00,000	10,000	14,000	3,500	1,69,500	15.62	5,48,700	3.23
T ₇	18,000	24,000	1,00,000	10,000	14,000	4,500	1,70,500	17	5,57,100	3.26
T ₈	18,000	24,000	1,00,000	10,000	14,000	1,46,350	3,12,350	70	8,10,000	2.59
T9	18,000	24,000	1,00,000	10,000	14,000	1,46,350	3,12,350	70	8,10,000	2.59
T ₁₀	18,000	24,000	1,00,000	10,000	14,000	74,925	2,40,925	86.59	8,85,000	3.67
T ₁₁	18,000	24,000	1,00,000	10,000	14,000	74,925	2,40,925	72.86	8,19,900	3.4

 $T_0 = Control$, $T_1 = Marigold leaf$, $T_2 = Cabbage leaf$, $T_3 = Vermicompost$, $T_4 = Biogas Digestate$, $T_5 = Bacterial suspension of$ *Bacillus subtilis* $, <math>T_6 = Bacterial suspension of$ *Pseudomonas fluorescens*, $T_7 = Rugby 10G$ (Positive Control), $T_8 = T_3 + T_5$, $T_9 = T_3 + T_6$, $T_{10} = T_4 + T_5$, $T_{11} = T_4 + T_6$; Tk = Bangladeshi Taka/currency; ha = hectare. * BCR was calculated on a variable cost basis.

suggested that application of cabbage leaves to control *M. javanica* is not a feasible option.

Vermicompost minimizes the use of chemical fertilizers and improves soil quality. Vermicomposts have abundant humic acid substances and hormones such as indole acetic acid (IAA), cytokinins, and gibberellins which could suppress nematode infestation (Oka, 2010). In this experiment, the individual application of vermicompost (T_3) did not have much impact on the reproductive parameters of M. javanica in both pot and field trials, although those were significantly lower than control (T_0) . Similarly, Mondal et al. (2021) observed that vermicompost exudate did not directly kill nematodes or alter the infectivity of J₂ in rice. There are published results that report a significant reduction in the numbers of nematode-induced galls with the application of vermicompost (Kumar et al., 2011; Xiao et al., 2016). According to Akhtar and Alam (1993), the effect of vermicompost on PPN depends on many factors, e.g., the type and ingredient of organic amendments, the dose, soil structure, nematode species, etc. Although reproductive parameters were largely unaffected by the individual application of vermicompost, growth parameters of the brinjal plants were found significantly higher than the control and some other treatments in this experiment. Kumar et al. (2011) found that a vermicompost fortification treatment resulted in increased growth characteristics with increases in sugar, protein, and lipid content. However, due to the requirement of a high amount of vermicompost to cultivate a unit area of land, production cost was higher for this treatment (T₃) leading to a lower BCR (1.73). Hence, the individual application of vermicompost might not be considered profitable for the control of RKN. Similarly, the results of this experiment do not support the application of BD to mitigate the infection by RKN, as this treatment (T₄) resulted in mixed results. It was found in this experiment that most of the reproductive parameters of RKN were significantly lower than the control (T_0) and vermicompost (T_3) , but higher than the positive control (T_7) in both the pot and field trial, and yield and BCR (2.37) were higher than vermicompost (T₃) for the individual application of BD (T₄). Ammonium and acetic acid ingredients of BD were proposed as the possible mechanisms of reducing PPN (Min et al., 2007) which is partly in agreement with the results of this experiment. However, the lower purchasing cost of BD contributed to the higher BCR than for vermicompost in this study.

In this experiment, two rhizosphere bacteria *B. subtilis* and *P. fluorescens* were applied individually (T_5 and T_6) and in combination with vermicompost and BD (T_8 , T_9 , T_{10} , and T_{11}) in both pot and field trials. In several preliminary *in vitro* experiments, it was found that *B. subtilis* and *P. fluorescens* strongly influenced the mortality and hatching of *M. javanica*, however, the pattern of influence of two organic amendments

(vermicompost and BD) on the nematode was inconsistent (Das et al., 2021a; 2021b). On the basis of these findings, organic amendments were integrated with the two bacteria in these trials. Different reproductive parameters (gall number, egg mass number, egg number, no. of J₂'s) were the lowest among all treatments and statistically similar to the positive control (T₇) for the individual application of both of the bacterial treatments in the pot trials. Similarly, Morgado et al. (2015) also observed that the nematicidal potential of B. subtilis was equivalent to that of the carbofuran treatments against M. incognita, M. javanica, and P. zeae in sugarcane. Borrajo et al. (2021) studied the biocontrol of the nematode M. javanica using several strains of P. fluorescens and Bacillus sp. and found that most of the tested strains reduced egg hatching and juvenile survival, however, juvenile mortality was higher when M. javanica was exposed to Bacillus spp. than to Pseudomonas spp. Berlitz et al. (2014) reported that B. subtilis reduced the number of eggs and J₂'s of *M. javanica* by producing antibiotics and hydrolytic enzymes which acted on the fecundity and fertility of the nematode. On the other hand, Pseudomonas spp. produce iron-chelating siderophores, antibiotics, and/or hydrogen cyanides that limit deleterious and pathogenic rhizosphere microorganisms (Dejene, 2014). In the pot trial, there were no egg masses, eggs, and J₂'s at 45 DAI, but they did occur at 90 DAI in the individual bacterial treatments (T₅ and T₆). This may have happened as some species of RKN undergo diapause under stressful conditions and resume activity when favorable conditions return (Wright and Perry, 2006). However, in the combined treatments (T₈, T₉, T₁₀, and T₁₁) reproductive parameters were higher than the individual application of bacterial suspension in pot trials, whereas, Rf was lower in the field. The microenvironment of a pot is different than the field and the mixing of two components might not have worked synergistically in that limited space. Both in the pot and field trials, growth parameters were statistically similar for the individual and combined treatments of bacterial suspension, whereas, the yield was significantly higher in the combined treatments compared to the individual bacterial treatments. Nath and Singh (2011) reported increased production of cauliflower after applying organic amendments with biopesticides which is in agreement with the findings of this study. Serfoji et al. (2010) tested the effectiveness of organic amendments and rhizotrophic rhizosphere microorganisms for the management of M. incognita on tomatoes. It was found that organic amendments in combination with Bacillus coagulans significantly increased the growth, biomass, and nutrients of tomatoes and decreased the RKN population and root-knot index. The use of organic amendments with biopesticides is helpful to compensate for the deficiency of nutrients in the soil as well as to

control PPN (Nath and Singh, 2011). Among the combined treatments, the combination of BD and B. subtilis (T_{10}) had the highest yield and BCR (3.67) with the lowest Rf. Among the plant growth-promoting rhizobacteria, Bacillus spp. has several benefits compared to other rhizobacteria. For example, Bacillus is omnipresent within the rhizosphere, endospore-forming, and highly resistant to heat, desiccation, and chemical destruction (Abu-Elgawad and Askary, 2018). In addition, BD contains considerable amounts of mineral elements (nitrogen, phosphorus, potassium) and organic matter that serves as a bio-fertilizer to stimulate crop yields (Koszel and Lorencowicz, 2015). Arshad et al. (2021) reported that the combined application of biochar and B. subtilis effectively managed RKN, enhanced overall plant biomass, and triggered defense-related genes in tomato plants. This finding supports the superior performance of the combined application of BD and B. subtilis obtained in this experiment, as both biochar and BD are produced by anaerobic digestion (Koszel and Lorencowicz, 2015).

In this work, several biorational components either individually or in combination were evaluated against infection of *M. javanica* of brinjal with regards to their efficacy and economic benefit. Results revealed that the combined treatments were better than the individual applications in terms of efficacy and profitability. The highest yield and BCR of brinjal were obtained by the combined application of biogas digestate and *B. subtilis* with the lowest R_f of *M. javanica*. To the best of our knowledge, this is the first report on a profitability assessment of BD in combination with a bio-agent in addressing the management of RKN which is important in the backdrop of the global concern for environmental pollution. However, further research on the ingredients of BD would be helpful to develop a more effective product. Brinjal cv. Noagram was found to be 'moderately resistant' and this could be a potential source of resistant genes against RKN.

Declarations

Author contribution statement

Sukalpa DAS: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Md. Abdul WADUD; Shila CHAKRABORTY: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Md. Atiqur Rahman KHOKON: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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