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Biofumigation potential of Indian mustard (*Brassica juncea*) to manage *Rhizoctonia solani*

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Abstract

In Egypt, *Rhizoctonia solani* is an economically important fungal pathogen on many crops such as common bean causing serious yield losses. Biofumigation with Indian mustard (*Brassica juncea*), as a potential alternative to the restricted fumigant methyl bromide, is gaining attention in sustainable vegetable production. In this study, laboratory and greenhouse experiments were conducted to evaluate the biofumigation effect of *B. juncea*, used as dry plants, seed meal, seed powder, methanol extract, and fresh plants (at the vegetative and flowering stages), against *R. solani*. Results showed that hexane defatted seed meal was the most efficient one, followed by the seed powder, fresh plants at the flowering stage then fresh plants at the vegetative stage. The fungal inhibition rate was 61.5, 50.2, 49.9, and 47.7%, respectively. While the dry plants at both flowering and vegetative stage recorded the lowest suppressive effect (44.3 and 39.1%, respectively). The findings open up the possibility of using the *B. juncea* in managing the root rot fungus, not only as a common green manure but also as a defatted seed meal.

Keywords: Biofumigation, Indian mustard, *Rhizoctonia solani*, Control

Background

Rhizoctonia solani is a soil-borne plant pathogenic fungus causing diseases on many economically important crops worldwide and is responsible for significant yield losses in a wide range of host plants, including agricultural and horticultural crops (Woodhall et al. 2007). *R. solani* caused a crop loss of 48% in stand establishment and 52% in seed yield of soybean (Handiseni et al. 2016). Chemical soil fumigant (i.e., methyl bromide) has been generally used to control the soil-borne pathogens. Despite methyl bromide efficiency in controlling a wide range of soil-borne plant diseases, this fumigant was phased out due to its ozone-depleting effect (Directive EC 128/2009) (Porter et al. 2010). Therefore, finding ozone friendly, safe, and sustainable alternative disease control option has become a necessity. Soil biofumigation was among the potential and suitable alternatives for disease management. "Biofumigation" is a term used to describe the suppression of soil-borne pests and pathogens by *brassica* species such as canola (*Brassica napus*) and Indian mustard (*Brassica juncea*) in rotation or as green manure crops (Wang et al. 2014). The use of Brassica crops as a biofumigant has been successfully exploited for the management of soil-borne pathogens and is growing and gaining interest. The biofumigation technique is managed in several countries at a full-field scale, e.g., USA, Australia, Italy, the Netherlands, and some others (Tollsten and Bergström 1988). *Brassica* crops contain significant quantities of the thioglucoside compounds known as glucosinolates (GSLs). When plants are incorporated into the soil, the plant tissues are ruptured allowing the GSLs and myrosinase enzyme come into contact and are hydrolyzed to release various forms of volatile isothiocyanates (ITCs) (Vig et al. 2009). ITC compounds are known to have broad pesticidal activity including insecticidal, nematocidal, fungicidal, antibiotic, and phytotoxic effects (Yulianti et al. 2006). The

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isothiocyanates produced by mustard are called "Allylisothiocyanate" (AITC), which is very similar to the chemical fumigant metam sodium. Controlling *R. solani* through biofumigation has shown varying success. *B. juncea* cultivars ("Brand 199," "Ruby Streak," "Florida Broadleaf," and "Green Wave") consistently provided > 90% mycelial inhibition in vitro for managing rice sheath blight caused by *R. solani* (Handiseni et al. 2016). In addition, AITC released from mustard was shown to be suppressive to *R. solani* in a controlled laboratory study (Charron and Sams 1999). Moreover, significant *R. solani* reduction was observed in greenhouse assays and also in field tests, following soil incorporation of brassica plant tissues, including *B. juncea* (Larkin and Griffins 2007). *B. juncea* was proved to be rich in ITCs and well-known in bioassay screenings of Brassicaceae cultivars as the most effective biofumigant (Hanschen and Winkelmann 2020). Therefore, the objective of the present work was to evaluate the biofumigation effect of the Indian mustard as an antifungal agent for controlling *R. solani* under laboratory and greenhouse conditions.

Materials and methods

Fungicidal effect of *Brassica juncea* under laboratory and greenhouse conditions

Seeds of *B. juncea* (cultivar Balady) were obtained from the commercial market, Cairo, Egypt. Different treatments were tested in the laboratory and in the greenhouse as follows: plant extract, seed powder (SP), hexane defatted seed meal (DSM), fresh plants at vegetative stage (FVS), fresh plants at inflorescence emergence stage (FIS), dry plants at vegetative stage (DVS), and dry plants at inflorescence emergence stage (DIS).

Plant materials and growth conditions

The mustard plant was sown and grown in pots (32 cm in diameter) under greenhouse conditions, 25 ± 2 °C, 75% relative humidity, and 16-h photoperiod. Two growth stages were sampled; after 4 and 8 weeks of sowing. Plants were harvested and washed to remove any adhering soil before dividing the plant samples into whole plants, shoots, and roots. The samples of different plant parts were kept under room temperature (25 ± 2 °C) for drying, then homogenized to a fine powder, and stored at − 20 °C for further experiments.

Inhibition of *R. solani* growth in the laboratory

Effect of Indian mustard methanol extract on the growth of R. solani

R. solani isolate was obtained from the Department of Vegetable Diseases at Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt. The extraction methodology of Doheny-Adams et al. (2017) was used with modification in which a total volume of 50 ml absolute methanol was added to 7.6 g finely ground plant samples. Methanol was added, at the two main growth stages: vegetative stage (VS) and inflorescence emergence stage (IS). The samples were then kept in a water bath at 40 °C for 10 min with shaking at 120 rpm and kept standing for 15 min before filtration through filter papers Whatman 1. The residue was re-extracted using the same procedure with combining the filtrates, and then methanol was evaporated using rotary evaporator under vacuum. The extract yield was weighed and sterilized, using a 0.45-μm syringe filter and series of dilutions were prepared and tested by Petri dishes bioassay.

Effect of Indian mustard fresh pieces on the growth of R. solani

To examine the inhibitory effect of the released compounds from the crushed tissues, four treatments were tested with four replicates each, in addition to the untreated control. The roots and the shoots were tested at the two growth stages, above-mentioned, according to Stephens et al. (1999). One gram of separated root and 2.5 g of separated shoots were crushed in mortar and pestle for 0.5–1.5 min then placed in upside-down position inside the lids of agar plates that contained 5 mm of actively growing *R. solani* culture and placed on the middle of the plate. Drops of water were added to the crushed plant tissues then the plates were immediately sealed with parafilm and kept in the dark at 25 ± 1 °C. Control treatment were prepared in the same manner but without the addition of the plant materials to the dish. The mycelial growth inhibition was calculated according to the formula of Lahlali and Hijri (2010) as follows:

(Control-Treatment)/Control*100. The radial growth was recorded by measuring the mean colony diameter when the fungus in the control plates reached the margin of the plate. This technique ensured that only volatile hydrolysis products formed by macerated *B. juncea* tissues contacted the fungus mycelium.

Inhibition of *R. solani* under greenhouse conditions

Preparation of R. solani inoculate and soil infestation

The tested fungus was grown on a sand-oatmeal sterilized medium and incubated at 25 °C for 2 weeks in the incubator. A ratio of 1:1 sand/clay soil was autoclaved for 20 min at 121 °C and repeated for 3 days intervals (Berns et al. 2008). A weight of 1 kg of soil was then infested with the fungus at 8% w/v. The artificially infested soil was then placed into cloth bags and moistened for one week to enhance the fungal growth.

Inhibitory effect of different treatments of Indian mustard

Infested soil was treated by the following treatments: SP, DSM, FVS, FIS, DVS, and DIS (Stephens et al. 1999; Salem and Mahdy 2015). These treatments were applied to the soil as weight/weight at 0.25, 0.5, 1, and 2%. The cloth bags, which contained infested soil with different treatments, were put inside plastic bags to prevent the loss of volatile hydrolysis products (due to damaged *B. juncea* tissues). The soil was moistened with the half field capacity prior to incubating for 7 days at 25 ± 2 °C. Two control treatments, healthy and infested with fungi, were prepared in the same manner but without adding mustard treatments. All treatments were replicated 4 times. After 2 weeks, germinated common bean seeds (*Phaseolus vulgaris*) were sown in pots (10 cm in diameter) with the rate of 2 seeds/pot and left for 20 days before recording the data. The following parameters were measured on the common bean seedlings:

(1) disease severity index: each plant was scored for damping-off severity and early root/hypocotyl damage using the 1–9 rating scale based on plant symptoms and root lesions (Peña et al. 2013), (2) plant fresh weight (g), and (3) the root and shoot length (cm).

Statistical analysis

Data were statistically analyzed by one-way ANOVA with the software SPSS. Variance homogeneity and the comparison between means were analyzed by Duncan's multiple range test (Duncan 1955).

Results and discussion

Laboratory studies

The extract of 7.6 g of dried plant materials with methanol yielded 2.17 g extract. The bioassay was then done as serial dilutions to determine the inhibitory effect of the plant under the study. The results revealed that the mustard extract reduced the mycelium growth of *R. solani* at the 2 growth stages, but IS (inflorescence emergence stage) extract was more effective than VS (vegetative stage) extract. As shown in Fig. 1, the effect was concentration-dependent as plant extract at 1/10 dilution (v/v) was the most effective, while 1/10000 dilution had the lowest inhibitory effect. Also, data illustrated in Fig. 2 showed that shoots and roots at VS reduced the fungal mycelial growth by 12.2 and 30.4%, respectively, Whereas shoots and roots at IS inhibited the growth by 57.1 and 82.2%, respectively. In general, roots were more effective than shoots, and IS was more effective than VS (Fig. 3). The methanol extract was used first to determine whether the plant has antifungal activity and to test which plant growth stage had more biological activity. Doheny-Adams et al. (I2017) highlighted that glucosinolates are highly polar compounds, but sensitive to the heat and are significantly degraded in temperatures ≥ 75°C in < 10 min. So in the present study, warm methanol was more preferable because it has a less hazardous effect and is more time- and cost-effective. So this technique preserves the volatile components, allowing the extract to exert its effect as a biofumigant against *R. solani*. The methanol extract revealed that the Indian mustard had inhibiting potential against *R. solani* at the two plant growth stages. It was clear that plants at the flowering stage had greater antifungal activity than the

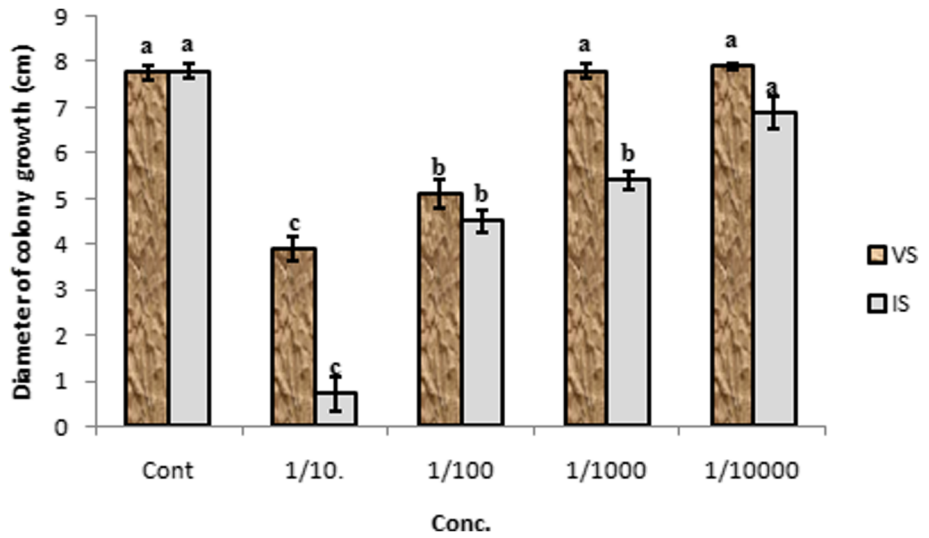

Fig. 1 Effect of mustard methanol extract on the growth of *R. solani* in the lab at two growth stages. *(VS) vegetative growth stage and (IS) inflorescence growth stage. Values have the same letter are not statistically different based on the Duncan's multiple range test at $p \leq 0.05$

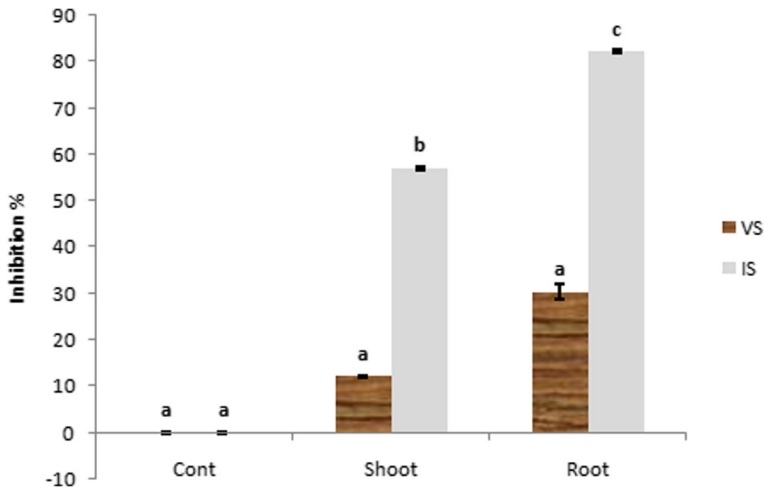

Fig. 2 Effect of mustard shoot and root pieces on the growth of *R. solani* in the lab at two growth stages. *(VS) vegetative growth stage and (IS) inflorescence growth stage. Values have the same letter are not statistically different based on the Duncan's multiple range test at $p \leq 0.05$

vegetative one. Several authors found similar results and recommended incorporation of some *Brassica* plants in the soil at the flowering stage (Stephens et al. 1999; Oliveira et al. 2011). Bellostas et al. (2004) attributed the differential efficacy during plant growth stages to the concentration changes of GSL (mainly sinigrin). Their study showed that when the plants reach the flowering stage, the GSL levels increased rapidly and concentrated in the reproductive organs. Additionally, the plant biomass reached its maximum during the flowering stage, which could provide more advantages for using mustard through that time. Obtained data examined the effect of different plant parts on the fungus at the lab level. There was a noticeable difference in activity between the root and the shoot parts, as root was more active than shoot. This finding is in agreement with Bellostas et al. 2004; Van Dam et al. 2009; Bhandari et al. 2015, and Villalta et al. 2016. Their studies revealed that, the root contains a high constant level of GSL during the plant life cycle, in contrast to the shoot which has a changeable concentration of GSL. This difference may be due to different factors such as genetics (Van Dam et al. 2009), environment, i.e., temperature (Sarwar and Kirkegaard 1998), and functional factors related to the defensive role of root against widespread soil pathogens. Second, the GSLs are classified as aliphatic, aromatic, or indole according to the side chain (R group). The different GSL types result in different ITCs products

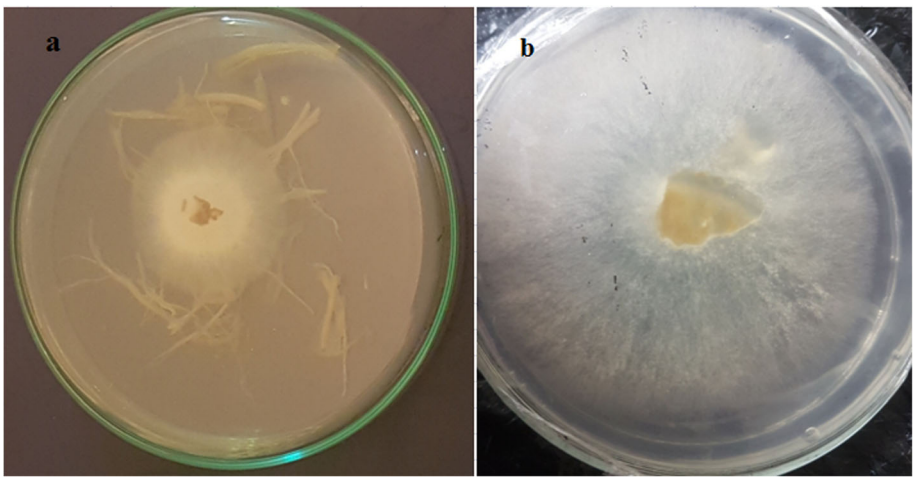

Fig. 3 Effect of mustard FIS root pieces on the growth of *R. solani* in the lab. **a** (FIS) fresh plants at inflorescence emergence, **b** control

responsible for the toxic effect. The GSL type differs severely within the plant species; it also varies among the different parts within the same plant (Bhandari et al. 2015). Moreover, it was found that the shoot contains predominantly sinigrin as an aliphatic GSL, while the root contains a complex of GLS: sinigrin (aliphatic) and gluconasturtiin (aromatic). This variation of GSL types in root endows it the detrimental impact against various microorganisms. The root is also well functionally adopted against vigorous pathogenic invasions, which are widely spread in the soil (Bhandari et al. 2015).

Greenhouse experiments

Greenhouse results revealed that incorporation of soil with all mustard treatments (SP, DSM, FVE, FIS, DVS, and DIS) at different concentrations (0.25, 0.5, 1, and 2%) reduced the fungal growth than the non-incorporated treatment. According to the disease severity index, obtained results showed that the defatted seed meal was the most effective treatment (Table 1). It inhibited the mycelial growth by 61.5%. While SP, FIS, FVE, DIS, and DVS had an inhibitory effect of 50.2, 49.9, 47.7, 44.3, and 39.1%, respectively. Also, it was observed that the inhibitory effect of different treatments was concentration-dependent, except for the dried plants, which showed opposite results as 2% DVS and DIS had the lowest effect (20.8 and 25.3%, respectively). The most effective concentration for DVS and DIS was 0.25 and 1%, which inhibited the fungus by 70.1 and 63.4%, respectively. The disease severity degree was in direct correlation with the plant fresh weight. The fresh weight of common bean seedlings treated with 2% of DSM, FIS, SP, and FVS was 100, 94.5, 81.3, and 52.3% compared to the healthy untreated control, respectively (Table 2). While this value at 0.25% of DSM, FIS, SP, and FVS was 55.7, 51.3, 51.5, and 21.5%, respectively. Also, it was observed that the length of the root and the shoot was clear and direct indicators for the plant development, which reflected on the fresh weight of the common bean (Figs. 4 and 5). Using DSM considered the most effective treatment, followed by FIS and SP, while dry materials had the lowest effect. These findings agree with Oliveira et al. 2011 and Michel 2014. Likewise, Shaban et al. (2011) indicated that mustard seed meal was the most effective treatment as it reduced the root rot and wilt disease incidence by 87.5 and 87.8%, respectively. Recent studies showed that using Brassicaceae seed meal alone or in combination with other techniques has promising results in controlling pre-plant diseases (Hanschen and Winkelmann 2020). On the other hand, Michel (2014) found that applying mustard hay in soil infested with *Verticillium dahlia* on tomato had no effect. Thus, the average root rot of tomato plants at the end of the trial had tremendously increased. He concluded that the effect of mustard hay was a long-term effect, and the number of *V. dahlia* microsclerotia was not influenced shortly after incorporation. Lazzeri et al. (2004) attributed the higher efficacy of the mustard seeds, and the DSM, specifically, to the high content of GSL than the other plant parts. Mustard seeds contain 35–40% oil (Anonymous 2019) which may bind to the GSL and subsequently prevent the enzymatic activity. But when the seeds are deoiled, the GSL become free from any bonds, and as a result, it has become available for the enzymatic reaction, and thus produces ITC. Another factor was discussed by Oliveira et al. (2011), which is the persistence time of ITC in the soil. Their study showed that the ITCs release rate and persistence time in the soil were very high in DSM, followed by SP. The present study on dried plant material showed that it had the least fungicidal effect which agrees with Lazzeri et al. 2004 who underlined the influence of the drying process on the GSL content. That probably led to the

Table 1 Effect of different mustard treatments with different concentrations (as percentage of the control) on the disease index of 20-days common bean seedlings infected with *R.solani*

Treat. %	Treatment											
	FVS		*FIS*		*DVS*		*DIS*		*SP*		*DSM*	
	M	%	M	%	M	%	M	%	M	%	M	%
I.Cont	6.7±0.3	100	6.3 ±0.3	100	6.7±0.3	100	6.3±0.3	100	6.7±0.3	100	6.7±0.3	100
0.25	5±0	25.3	4.3±0.3	31.7	2±0	70.1	4±0	36.5	4.3±0.3	35.38	4±0	40.2
0.5	3.3±0.3	50.7	3.3 ±0.3	47.6	4±0	40.2	3±0	52.3	3.7±0.3	44.7	3±0	55.2
1	3±0	55.2	3 ±0	52.3	5±0	25.3	2.3±0.3	63.4	3±0	55.2	2±0	70.1
2	2.7±0.3	59.7	2±0	68.2	5.3±0.3	20.8	4.7±0.3	25.3	2.3±0.3	65.6	1.3±0.3	80.6
AVG	**47.7%**		**49.9%**		**39.1%**		**44.3%**		**50.2%**		**61.5%**	

The values are expressed as mean ± standard error

M mean, *I.Cont* Infested control, *FVS* fresh plants at vegetative stage, *FIS* fresh plants at inflorescence emergence, *DVS* dry plants at vegetative stage, *DIS* dry plants atinflorescence stage, *SP* seed powder, *DSM* hexane defatted seed meal

Table 2 Effect of different mustard treatments with different concentrations (as a mean and a percent of the control) on the fresh weight of 20-days common bean infected with *R.solani*

Conc. %	Treatment											
	FVS		*FIS*		*DVS*		*DIS*		*SP*		*DSM*	
	M	%	M	%	M	%	M	%	M	%	M	%
Cont.	6.5±.0	100	3.7±.0	100	6.1±.3	100	3.4±.1	100	6.4±.0	100	6.1±.0	100
0.25	1.4±.0	21.5	1.9±.3	51.3	3.6±.1	59	2.6±.0	76.4	3.3±.2	51.5	3.4±.3	55.7
0.5	2.4±.1	36.9	2.4±.2	64.8	3.8±.1	62.2	2.9±.1	85.2	3.3±.7	51.5	4.6±.2	75.4
1	3.3±.2	50.7	2.9±.1	78.3	3.8±.1	62.2	3.1±.1	91.1	4.2±1.3	65.6	5.4±.1	88.5
2	3.4±.2	52.3	3.5±.2	94.5	3.1±.2	50.8	2.3±.0	67.6	5.2±.03	81.3	6.1±.1	100

The values are expressed as mean ± standard error
M mean, *FVS* fresh plants at vegetative stage, *FIS* fresh plants at inflorescence emergence, *DVS* dry plsnts at vegetative stage, *DIS* dry plants atinflorescence stage, *SP* seed powder, *DSM* hexane defatted seed meal

glucosinolates leakage and myrosinase activity loss, which results in decreasing the efficacy.

In general, *Brassicas* particularly mustard revealed a potential benefit in controlling root rot disease, where the use of methyl bromide has been banned. In this regard, Lord et al. (2011) pointed out that the biocidal activity of the glucosinolates released from *B. juncea* is comparable with the efficacy of chemical pesticides and antibiotics. Accordingly, synthetic pesticides, such as methyl bromide, could be replaced by *Brassica* plants (Rokunuzzaman et al. 2016).

Conclusion

Biofumigation with Indian mustard can be exploited in soil fumigation in different methods particularly, as fresh plants in the common green manure, and seed meal after oil extraction based on the laboratory and greenhouse conditions. Further studies are needed to assess

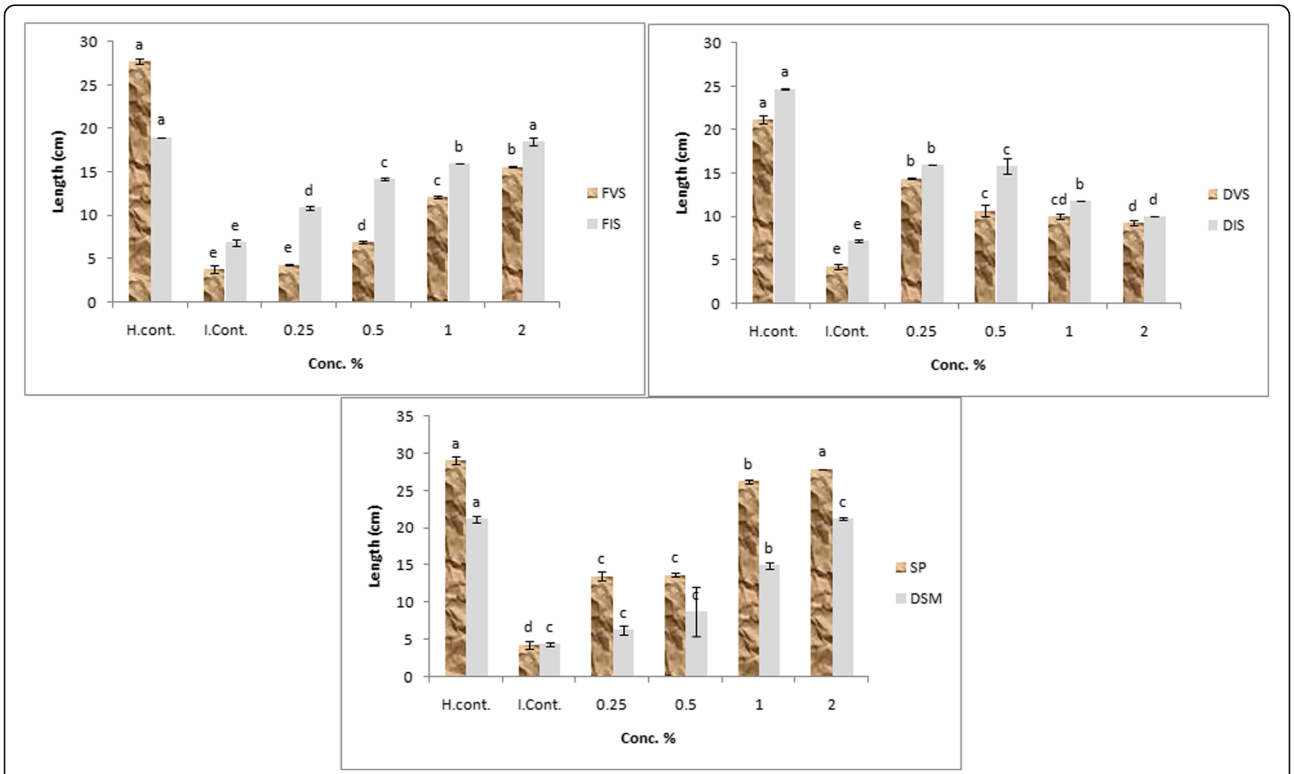

Fig. 4 Effect of different mustard treatments with different concentrations on the root length of 20 days common bean infected with *R. solani*. (H.cont) healthy control, (I.cont) infested control (FVS) fresh plants at vegetative stage, (FIS) fresh plants at inflorescence emergence, (DVS) dry plants at vegetative stage, (DIS) dry plants at inflorescence stage, (SP) seed powder, and (DSM) hexane defatted seed meal. Values have the same letter are not statistically different

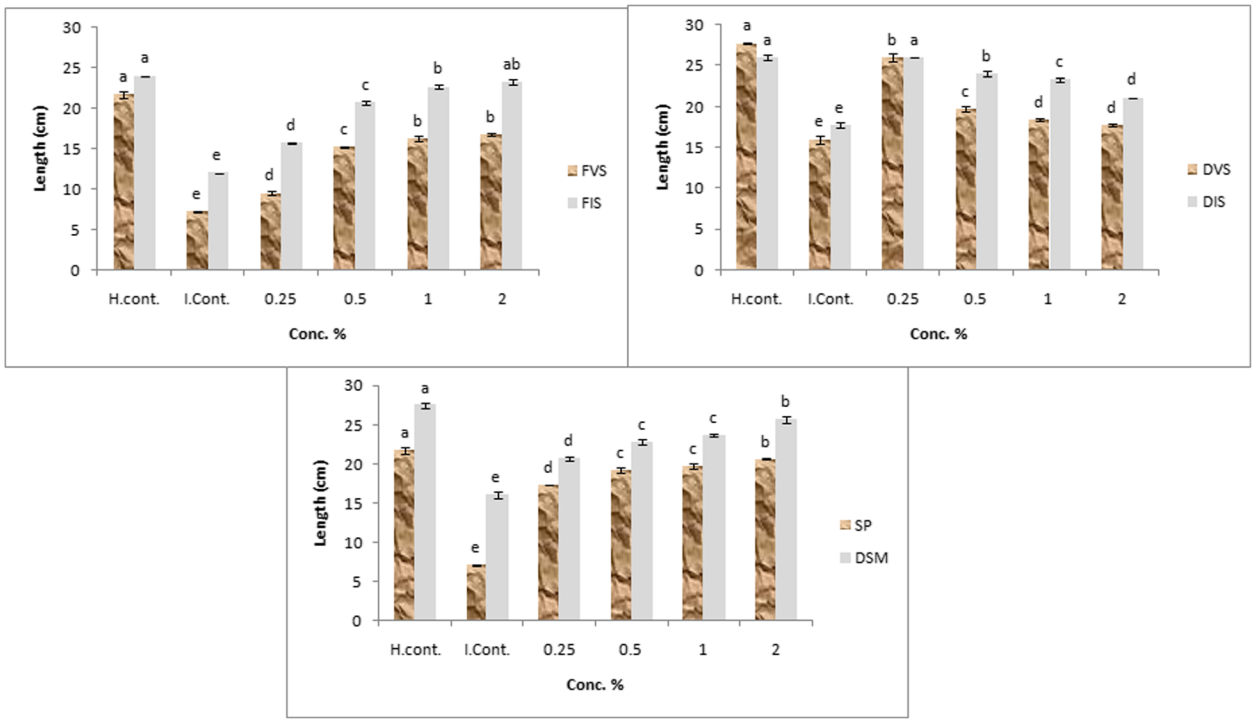

Fig. 5 Effect of different mustard treatments with different concentrations on the shoot length of 20 days common bean infected with *R. solani*. (H.cont) healthy control, (I.cont) infested control (FVS) fresh plants at vegetative stage, (FIS) fresh plants at inflorescence emergence, (DVS) dry plants at vegetative stage, (DIS) dry plants at inflorescence stage, (SP) seed powder, and (DSM) hexane defatted seed meal. Values have the same letter are not statistically different

the biological activity of this promising plant under natural field conditions. These first results open new perspectives for the application of biofumigation in plant protection and management. Biofumigation has advantages over other disease control methods, since it is used to reclaim soils contaminated with heavy metals and adds organic matter to the soil. Hence, what gives an advance for biofumigation is its ability to work as biopesticide and simultaneously as a soil-improvement tool. Farmers should be aware of the usefulness of this technique, in order to be implemented in their farming systems.

Abbreviations

DIS: Dry plants at inflorescence emergence stage; DSM: Hexane defatted seed meal; DVS: Dry plants at vegetative stage; FIS: Fresh plants at inflorescence emergence stage; FVS: Fresh plants at vegetative stage; IS: Inflorescence emergence stage; SP: Seed powder; VS: Vegetative stage

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Not applicable for this study.

Authors' contributions

All authors designed the experiments. IA and MAK supervised and coordinated the laboratory work, results analysis, and manuscript drafting. RY performed the experiments and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data and material used during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Ethical approval and consent to participate are not required for this study.

Consent for publication

Not applicable for that section.

Competing interests

The authors declare that they have no competing interests.

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