EFFICACY OF SNEB183 (*SINORHIZOBIUM FREDII*) AGAINST SOYBEAN CYST NEMATODE (*HETERODERA GLYCINES*) UNDER FIELD AND GROWTH CHAMBER CONDITIONS

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Abstract. Soybean cyst nematode (*Heterodera glycines*) has become a serious risk for soybean production globally. Chemical nematicides pose a severe threat to human health and also pollute the environment whereas, biological control is a safe and eco-friendly method used to control pathogens. The aim of this study was to discover novel biocontrol agents against *H. glycines* by coating soybean seeds with biocontrol agent Sneb183 (*Sinorhizobium fredii*) and to assess its potential to control this pest. The stability and efficiency of Sneb183 (*S. fredii*) to control *H. glycines* in field and growth chamber experiments were investigated. Seed coating with Sneb183 significantly decreased the presence of *H. glycines* by 35.58%, 64.09% and 50.43% in experiments J2 (second-stage juveniles), J3 (third-stage juveniles) and J4 (fourth-stage juveniles), respectively as compared to control. A progressive increase in the concentrations of the Snef183 resulted in a significant reduction of *H. glycines*. Additionally, Sneb183 boosted seed germination and seed vigor index. Moreover, seed coating with Sneb183 increased plant biomass. These results demonstrate that Sneb183 (*S. fredii*) is a promising biocontrol agent against *H. glycine* and biomass promoter of soybean.
Keywords: *pathogen, biocontrol, seed germination, vigorous index, biomass*

Introduction

Soybean (*Glycine max* L.) is a chief source of food and oil products throughout the world. Plant-parasitic nematodes (PPN) can simply destroy oil crops by feeding and also facilitates the spread other pathogenic organisms such as bacteria, viruses and fungi (Palomares-Rius et al., 2017; Sikandar et al., 2020a). Some of them are obligate parasites in nature whereas, others are sedentary such as root-knot nematodes of the genus *Meloidogyne* and cyst nematodes of the genera *Heterodera* and *Globodera* (Kihika et al., 2017; Palomares-Rius et al., 2017).

Soybean cyst nematode (*Heterodera glycines*) has adversely affected growth and seed yield of soybean globally (Rincker et al., 2017). It causes an enormous threat to soybean production, manifested in up to 1.5 billion USD global losses and 120 million USD worth of damage in China annually (Moens et al., 2008; Hosseini and Matthews, 2014). Synthetic chemical nematicides have been applied to manage nematodes but their haphazard uses cause serious consequences on human's health and environment (Sikandar et al., 2020b). That's why several chemical nematicides have been banned in

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different countries (Nicol et al., 2011). With increasing demands of safe and eco-friendly strategies for management and prevention of *H. glycines* are needed urgently.

Recently, the applications of biocontrol agents are capable of controlling *H. glycines*. Biological control is generally safe as compared to synthetic chemical control. There is the richness of soil-borne microbes in rhizosphere soil like *Sinorhizobium* (Tian et al., 2014), *Pseudomonas* spp. (Prabhukarthikeyan et al., 2018; Meena et al., 2019) *Bacillus* spp. (Zhao et al., 2018; Beeman and Tylka, 2018) and *Burkholderia* spp. (Ji et al., 2010; Kurepin et al., 2015) these are valuable for plant growth.

Several microbial strains have antagonistic potential toward plant-parasitic nematodes (Gao et al., 2016; Zhai et al., 2018; Xiang et al., 2018). Soybean seed treated by *B. simplex* effectively reduced *H. glycines* by triggering induced systemic resistance (Xiang et al., 2013; Xiang et al., 2016). Zhao et al. (2019) also reported that soybean seeds treated with Sneb159 (*Microbacterium maritypicum*) reduced the *H. glycines* in field and growth chamber experiments. Sneb183 (*Sinorhizobium fredii*) was isolated from nodules of soybean and efficiently decreased *H. glycines* by reducing oxygen consumption of J2s (Tian et al., 2014; Wang et al., 2020). Seed coating with plant growth-promoting bacteria is an effective and simple technique against pathogens (Pathak et al., 2016).

Therefore, keeping in view the biocontrol ability of Sneb183 (*Sinorhizobium fredii*) toward *H. glycine*, the study was aimed to explore the evaluation of Sneb183 (*Sinorhizobium fredii*) on growth of soybean and control of *H. glycine* by seed coating in field and growth chamber. The outcomes of the current study should help in the progression of valuable and marketable biocontrol agents.

Materials and Methods

Isolation of cysts and J2 of SCN

Soil samples from 2 cm depth were obtained from the rhizosphere of soybean (*Glycine max* cv Liaodou 15, a SCN-susceptible cultivar) grown in greenhouse of Nematology Institute of Northern China (NINC), Shenyang Agricultural University, China. Cysts were collected through hand-picking under a stereomicroscope (Nikon SMZ800, Nikon, Tokyo, Japan) and were surface-sterilized by 0.1% $HgCl_2$ solution for 1 minute and then rinsed three times with distilled water for the complete elimination of $HgCl_2$. The cysts were shifted in Baermann funnel at 25℃ and J2s were collected every two or three days (Liu, 1995).

Effect of Sneb183 on the seedling growth of soybean

Sinorhizobium fredii Sneb183 preserved at -80℃ was suspended in sterilized water and adjusted to 1.0×10^8 cfu/ml with a hemocytometer under a microscope. The medium was sterilized in steam autoclave machine (Zealway (Xiamen) Instrument Co., Ltd. Model no.GI54DS) for 30 min at 121℃. The 1.0 mL of suspension was poured to 50 mL sterilized TY liquid medium (Duelli and Noel, 1997). Cultures were maintained at 28℃ and 150 rpm for 168 h in a shaker (ZWY-1102C incubator shaker), the fermentation broth was labelled as Sneb183F.

Soybean seeds Liaodou15 were used in all the experiments. Seeds were surface-sterilized with 0.5% NaOCl for 10 min and then washed several times with sterile distilled water and air-dried (Hosseini and Matthews, 2014). Seeds were equally coated with Sneb183F with a ratio of 70:1 (g/ml) and distilled water was used as a control treatment

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(Zhou et al., 2017). The coated seeds were transferred into the petri dish contained wet filter paper and incubated at 28 ± l℃ for one week (Fan et al., 2017; Sikandar et al., 2019). Germination of seeds was counted on a daily basis. After 7 days of incubation, the radicles were cut off, and the fresh weight of radicles per 10 seeds was calculated. The average germination percentage ± SE (standard error) of triplicate experiments was calculated. Seedling vigour index (VI) was determined by using the *Eq.1* described by Abdul-Baki and Anderson (1973).

$$\text{Vigor index (VI)} = (\text{M.S.L} + \text{M.R.L}) \times \text{G\%} \quad \text{(Eq.1)}$$

whereas; M.S.L (Mean shoot length); M.R.L (Mean root length); G% (Germination percentage).

Biocontrol efficiency of Sneb183 against H. glycines in growth chamber experiments

Coated seeds with 1x, 5x and 10x diluted Sneb183 fermentation filtrate were planted in pots (25 cm in diameter) with sterilized soil and sand (2:1) and irrigated on alternate days with N-free Hoagland's solution, in which KNO_3 and $Ca(NO_3)_2$ were substituted with KH_2PO_4 and $CaCl_2$ (Hoagland and Arnon, 1950). Plants were placed in the illuminated chamber (16-h photoperiod and 23°C–26°C temperature). At the second true leaf stage, 2 ml freshly hatched J2s (approximately 1,500) were inoculated into two 1-cm-deep holes around the stem base by pipetting. The number of juveniles was observed after 7 days post-inoculation (dpi) by acid fuchsin staining and mature females were calculated at 35 dpi. The experiment was conducted in a completely randomized block design, and all growth chamber treatments were replicated eight times.

Biocontrol efficiency of Sneb183 against H. glycines in field experiments

Field experiments were conducted at Kangping County (Liaoning Province, China) during 2018. Each experiment comprised of two treatments: Sterilized seeds coated with Sneb183 as treated and with distilled water as the control. All treatments were arranged in a randomized complete block design with five replications. Experimental plots were 7-m long, 3.5-m wide and contained 6 rows (70 seeds per row). After 35 days, 12 seedlings were randomly selected by using the Z-shaped sampling method. Seedlings were carefully removed and the number of females was counted immediately on the roots. 100 ml of rhizosphere soil samples were randomly collected for experimental plots. The females in the soil were isolated and counted as described above. The length of shoots and roots, the root weight and fresh weight of whole plants were recorded for all sixty seedlings per treatment. Then, the number of juveniles inside the roots was determined in twenty-five seedlings and the dry weight of the plants seedlings were measured from thirty-five other seedlings. Randomly selected plants were evaluated for plant height, number of pods and seeds per plant. Seeds were threshed, and 100-seed weight was measured four times per plot.

Effect of Sneb183 on the development of soybean cyst nematode (SCN) in soybean roots

Plants were inoculated with J2s of SCN as described above. Two days after inoculation, plants were transferred to new pots and effect of Sneb183 on the development of SCN in soybean root was determined. Roots samples were collected at 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 days after the nematode inoculation. For each time interval, six

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plants were randomly selected. Roots were carefully removed from pots, washed gently and weighed. The clean roots were stained with 0.1% acid fuchsin solution. The distribution of the different developmental stages, namely J2, J3, J4 and adults were calculated under a stereomicroscope. The entire experiment was repeated twice.

Effect of Sneb183 on J2s Infiltration of Soybean Root Position

After 3 days of inoculation of J2, the root system of soybean was carefully removed and stained. Then, the number of J2s was observed under stereomicroscope. According to the distance of 0.5 cm from the root tumor as a distance unit, the number of invasion of J2s at different distance units was calculated.

Statistical analysis

In order to assess the significant effects, data was analyzed by using one-way analysis of variance (ANOVA). Means difference for each trial was calculated by the Duncan multiple range test. All statistical procedures were directed by IBM-SPSS statistics 25.0 version software and graphs were built on Sigma Plot 10.0 software.

Results

Effect of Sneb183 on the seedling growth of soybean

The effect of fermentation on the seedling growth of soybean is shown in *Fig. 1* and *Fig. 2*. The significant difference ($P<0.05$) was observed in coated seeds with fermentation broth of Sneb183 which boosted the germination up to 92%. Fresh and dry weight of radicles demonstrated the potential of Sneb183 which increased to 9.63% and 19.23%, respectively as compared to control. The mean root length, mean shoot length and vigor index were significantly boosted up to 29.33%, 21.55% and 39.33%, respectively by seed coating with *Sinorhizobium fredii* (Sneb183).

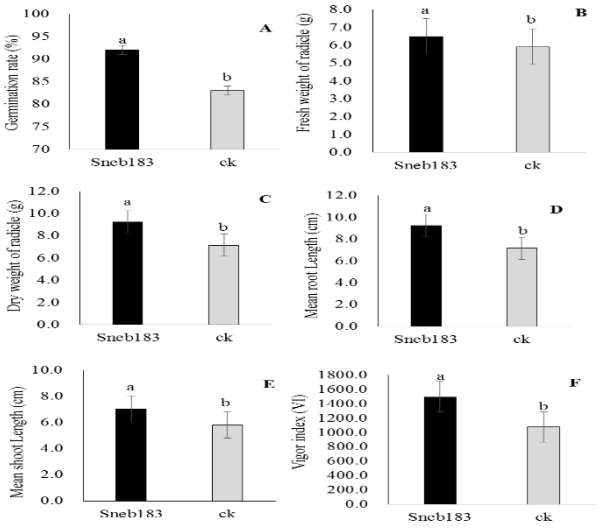

Figure 1. *Effect of Sneb183 on the seedling growth of soybean. (A) Germination percentage of soybean seeds. (B) Fresh weight of radical. (C) Dry weight of radicals. (D) Mean root length. (E) Mean shoot length. (F) Vigour index of treated and untreated seeds. Different letters on bar indicates that values are significantly different according to Duncan's multiple range test at P>0.05*

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Figure 2. *Efficiency of coating with Sneb183 on the germination of soybean seeds*

Biocontrol efficiency of Sneb183 against H. glycines in growth chamber experiments

The *Sinorhizobium fredii* Sneb183 was tested for its potential against *H. glycines* in growth chamber experiments. The plants were monitored regularly to examine the number of juveniles at 7 dpi and mature females at 35 dpi.

Table 1 demonstrated that all the treated seed with different dilution of fermentation Sneb183 efficiently reduced the invasion of J2s and development of matured females. However, nematicidal potential was directly related to the concentration of fermentation of Sneb183. The maximum potential of Sneb183 was observed in 1x dillution because less number of juvenlies and females recored in this dillution as compared to 5x and 10x dilution. A progressive increase in the concentrations of the Sneb183 resulted were significantly reduced *H. glycines*.

Table 1. *Effects of Sneb183 at different dilution on H. glycines in growth chamber experiments*

Treatments	1x	5x diluted	10x diluted	CK
Juveniles	91.1±13.09	98.9±20.49	107.4±15.85	151.8±25.27
Females	62.4±9.67	70.4±11.33	78.4±17.21	119.4±26.37
ANOVA Test				
S.S	4118.45	4061.25	4205.0	5248.80
df	1	1	1	1
M.S	4118.45	4061.25	4205.0	5248.80
F	31.08	14.82	15.36	7.87
P	0.000	0.001	0.001	0.012

Data represent the Mean±Standard deviation. Whereas; S.S (Sum of square); M.S (Mean square); df (Degree of freedom); F (F-value); P (significant value)

Biocontrol efficiency of Sneb183 against H. glycines in field experiments

To determine the biological control effect of Sneb183 on *H. glycines* and growth parameters were evaluated under field conditions. The plant height and shoot length were increased by Sneb183 (*Table 2*). Sneb183 significantly reduced the number of cyst on the roots up to 27.14% as compared with the control. The number of seeds and number of pods were increased in Sneb183-treated plants (*Table 3*). It is clear from outcomes of field experiment that the Sneb183 displayed a drastic decrease in no. of cysts (*Table 2* and *Table 3*).

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Table 2. *The growth condition of soybean and cyst number on soybean roots in field*

Treat.	Shoot length (cm)	Plant height (cm)	Root length (cm)	Fresh weight (g)	Root weight (g)	Cyst
Sneb183	24.7± 5.6	100.1±12.4	21.8±4.3	7.8±0.93	1.5±0.25	63.8±8.2
Control	21.7 ±2.8	96.6±11.20	17.7±2.8	5.3±0.85	1.2±0.29	116±13
ANOVA Test						
S.S	45.36	61.25	87.65	31.53	0.61	13833.80
df	1	1	1	1	1	1
M.S	45.36	61.25	87.65	31.53	0.61	13833.80
F	2.35	0.44	6.48	40.14	8.4	111.36
P	0.14	0.517	0.020	0.000	0.009	0.000

Data represent the Mean±Standard deviation. Whereas; S.S (Sum of square); M.S (Mean square); df (Degree of freedom); F (F-value); P (significant value)

Table 3. *Effects of rhizobium Sneb183 on soybean yield*

Treat.	Pot number per plant	Seed number per plant	100-seed weight (g)
Sneb183	46.30±5.03	133.20±7.67	26.42±2.98
Control	36.70±3.06	97.40±7.32	24.74±2.25
ANOVA Test			
S.S	344.45	6408.20	14.03
df	1	1	1
M.S	344.45	6408.20	14.03
F	26.04	113.98	2.01
P	0.000	0.000	0.173

Data represent the Mean±Standard deviation. Whereas; S.S (Sum of square); M.S (Mean square); df (Degree of freedom); F (F-value); P (significant value)

Effect of Sneb183 on the development of SCN in soybean roots

The Sneb183 was verified for its ability to control on the development of SCN in soybean roots. Plants were examined frequently to observe the development of soybean cyst nematode at 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 days after inoculation. Data in *Fig. 3* and *Fig. 4* presented that Sneb183-treated plant efficiently inhibited the invasion of J2s into soybean roots and reduced the invasion up to 35.58%. It also exhibited the development of nematodes J3 and J4 and reduced the development up to 64.09% and 50.43%, respectively. In comparison, at all dpi-s control seedling had maximum population of SCN but, Sneb183-treated plants displayed significantly less nematodes.

Effect of Sneb183 on J2s Infiltration of Soybean Root Position

The effect of *S. fredii* Sneb183 on the infiltration of J2 in soybean root was calculated. The invasion of J2 infiltration at different distance units from the root tumor was represented in *Figure 5*. The maximum J2 were attracted at 2 cm whereas their minimum attraction was observed at 0.5 cm and 4 cm distance units from the soybean root tumor.

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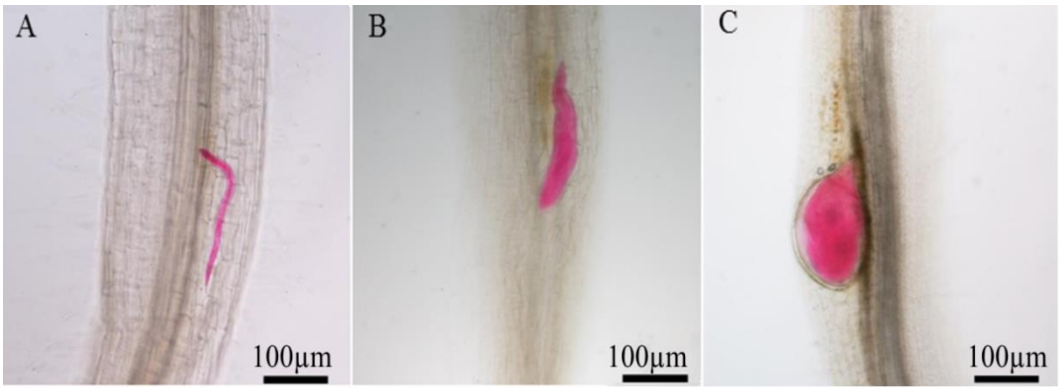

Figure 3. *Development of SCN in soybean roots. (A) J2 in the root system. (B) J3 in the root system. (C) J4 in the root system. These pictures were taken in compound microscope (Olympus bx53 compound microscope, Olympus Co., Tokyo, Japan) and Each scale bar = 100 μm*

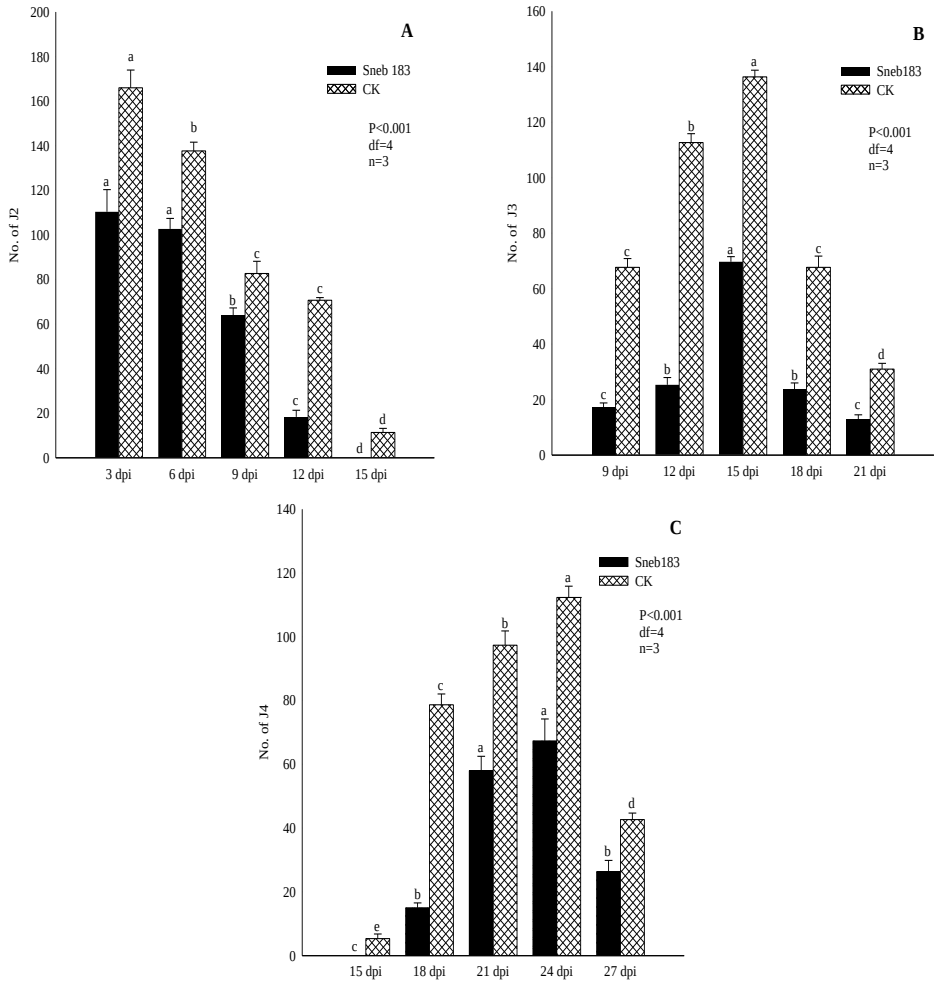

Figure 4. *Effect of Sneb183 on the development of SCN in soybean roots. (A) Number of J2 in the root system. (B) Number of J3 in the root system. (C) Number of J4 in the root system. Different letters on bar indicates that values are significantly different according to Duncan's multiple range test at P>0.05*

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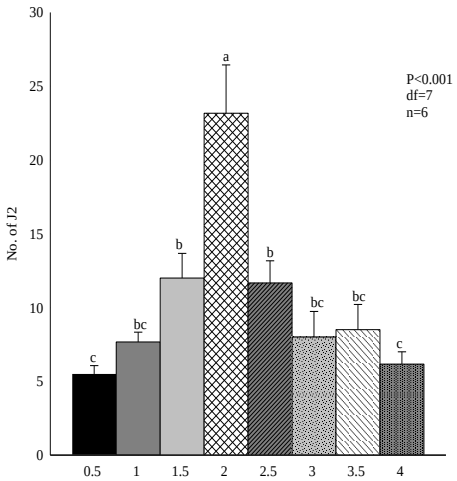

Figure 5. *Efficacy of Sneb 183 (Sinorhizobium fredii) on J2 Infiltration of Soybean Root Position. Different letters on bar indicates that values are significantly different according to Duncan's multiple range test at P>0.05*

Discussion

Soybean is an essential legume but it is threatened by soybean cyst nematode (*H. glycines*). Plant growth promoting bacteria are very effective biocontrol agents in current agriculture (Kumari and Sivakumar, 2005; Séry et al., 2016). In present study, soybean seeds coating with Sneb183 effectively delay the development of juvenile and also suppressed the number of adult females of *H. glycines* as compared to control in growth chamber experiment. Liu et al. (2018) also supported our finding by explaining that seed coating with SnebYK (*Klebsiella pneumoniae*) had capability to control development of juvenile and adults of *H. glycines*. Zhou et al. (2017) described that the *Sinorhizobium fredii* Sneb183 significantly promoted plant growth and reduced soybean cyst nematode. The outcomes showed that seed coating improved biomass and also reduced nematodes infection. Thus, our results are agree with Kang et al. (2018) who reported that the seeds coating with Sneb545 *B. simplex* promoted resistance in soybean toward *H. glycines*. The findings of Zhao et al. (2019) are in agreement with our findings that seeds treated with Sneb159 *M. maritypicum* has the potential toward *H. glycines* in field and growth chamber experiments.

Seed coating with plant growth-promoting bacteria enhanced germination of seeds. Seed dressing with fungus and bacteria displayed enhanced germination, seedling vigor and reduced nematodes infection (Zhao et al., 2018; Sikandar et al., 2019). Seed coating with Sneb183 is a reasonable and effective way to boosted the germination of seeds and reduce invasion of nematodes in soybean roots.

Rhizobium-legume interaction is a mutual relationship which is important symbionts that provide nitrogen to soybean (Coba de la Peña et al., 2018). Sneb183 significantly enhanced seedling growth, particularly that of the root of soybean. Non-symbiotic nitrogen fixing bacteria provide nitrogen and increasing growth of plant (Hayat et al., 2010). They particularly increase weight (Singh et al., 2017) and root surface area of plant (Wang et al., 2015).

Soybean is an excellent and susceptible host of *H. glycine* however, seeds coating with Sneb183 expressively enhanced biomass and reduced the nematode infection in soybean.

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Although, many methods have been used in modern agriculture to control *H. glycine*, but implication of seed coating with Sneb183 is limited to a fewer study. The results of present study demonstrated that Snef183 is beneficial for seed coating, in order to promote plant growth and reduced nematode invasion and development. These consequences presented that seed coating is an effective and reasonable alternative approach to control *H. glycine*.

Conclusion

It is concluded that Sneb183 have nematicidal potential toward soybean cyst nematode and also presented remarkable growth-promoting characters in both field and growth chamber experiments in view of our findings. Current outcomes could provide a foundation for *S. fredii* (Sneb183) as a biocontrol agent for soybean cyst nematode (*H. glycine*). It can be considered as a commercial biocontrol agent, however, before commercial recommendation further studies are desired to evaluate its active component screening and mechanisms of action.

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Conflict of interests. All authors have no conflict of interests.

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