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Compatibility studies between different bacterial and fungal biocontrol agents and neem cake for management of root knot nematode, *Meloidogyne incognita* in okra

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Abstract
The present study was undertaken to find compatible nature between different biocontrol agents such as *Bacillus firmus, Bacillus subtilis, Trichoderma asperellum, Purpureocillium lilacinum* along with organic amendment neem cake. The results exhibited that among different bacterial biocontrol agents *B. firmus, B. subtilis* were found to be compatible with each other and fungal biocontrol agents *Trichoderma asperellum, Purpureocillium lilacinum* were found to be compatible with each other whereas response for fungal and bacterial biocontrol agents *B. firmus B. subtilis* were found to show incompatible reaction with *P. lilacinum* and in contrast the compatible reaction was observed between *B. firmus, B. subtilis, T. asperellum*. The findings also stated that all the biocontrol agents were found to be compatible with neem cake.

Keywords: biocontrol agents, neem cake, compatibility, incompatibility, *Bacillus firmus, Bacillus subtilis, Purpureocillium lilacinum, Trichoderma asperellum*

Introduction
Root knot nematode, *Meloidogyne incognita* threatens the okra production across the globe by limiting the production around 14 percent in open field conditions and 60 percent under protected cultivation (Gowda *et al*., 2019) [4]. Symptoms of okra plants infested with *M. incognita* exhibits the profused root galls as below ground symptoms and stunting, yellowing as above ground symptom. Usage of chemical nematicides not only affects the environment but also the consumers intaking the nematicide residual food substances which may lead to serious health disorders Hartman and Sasser (1985) [5]. Focus must be shifted for developing the safe nematode management techniques which helps to supress the nematode population below the threshold level with low input cost. Though the biocontrol agents were found to be effective against root knot nematodes, *M. incognita* under solo application the combined effect of different biocontrol agents will ensure maximum plant growth parameters with minimum nematode population and input cost. Hence the present study was undertaken to study the compatibility reaction between bacterial and fungal biocontrol agents along with neem cake under *in-vitro* conditions to develop an Integrated nematode management strategy against *M. incognita.*

Materials and Methods
Compatibility study on biological control agents with neem cake
Ten gram of neem cake was pre-soaked in 100 ml of distilled water over night. The extract was filtered using Whatman No.1 filter paper and centrifuged at 6500 rpm for 15 minutes. The supernatant was further filtered using 0.2µ bacteriological filter and 10 percent concentration of extract was added to PDA and LB medium. The 8 mm fungal disc was inoculated and bacterial biocontrol agents were streaked in medium containing neem cake extract.

All the plates were incubated at 28±1 ^{0}C and observed for zone of inhibition (if any). Presence of inhibition zone indicates the incompatibility of biocontrol organisms and vice versa. The experiment was carried out by following Completely Randomised Design (CRD). Treatment details were as follows, T_1 – *Bacillus firmus* + neem cake, T_2 – *Bacillus subtilis* + neem cake, T_3 – *Trichoderma asperellum* + neem cake, T_4 – *Purpureocillium lilacinum* + neem cake, T_5 – *Bacillus firmus* alone, T_6 – *Bacillus subtilis* alone, T_7 – *Trichoderma asperellum* alone, T_8 – *Purpureocillium lilacinum* alone.

Compatibility study between different biocontrol agents

Compatibility between bacterial biocontrol agents *B, firmus* and *B. subtilis* was done by method of Fukui *et al*. (1994) [3]. Streaking the cultures horizontally and vertically in a plate having nutrient agar medium. For fungal biocontrol agents, 5mm disc of *T. asperellum* was inoculated at one cm away from the periphery of plate and 5m disc of *P. lilacinum* were inoculated at opposite side in plates containing potato dextrose agar medium.

For studying compatibility between fungal and bacterial biocontrol agents procedure of Dennis and Webster (1971) [2] was followed. The plates containing potato dextrose agar medium were inoculated with 5mm disc of fungal bioagents in one cm away from periphery of plates and bacterial culture was streaked on opposite end. The plates containing individual culture served as control. Treatment details were as follows, T_1 – *Bacillus firmus* + *Bacillus subtilis*, T_2 – *Bacillus firmus* + *Trichoderma asperullum* T_3 – *Bacillus firmus* + *Purpureocillium lilacinum* T_4 – *Bacillus subtilis* + *Trichoderma asperullum*, T_5 – *Bacillus subtilis* + *Purpureocillium lilacinum*, T_6 – *Trichoderma asperullum* + *Purpureocillium lilacinum*, T_7 – *Bacillus firmus* alone, T_8 – *Bacillus subtilis* alone, T_9 – *Trichoderma asperullum* alone, T_{10} – *Purpureocillium lilacinum* alone.

Results and Discussion

Compatibility studies on different biocontrol agents with neem cake

The results of compatibility studies on different biocontrol agents with neem cake showed that *B. firmus, B. subtilis, T. asperellum, P. lilacinum* had good growth in plates amended with neem cake. The results stated that all the biocontrol organisms were compatible with neem cake. (Figure 1, Table 1). The present findings were in correlation with Zope *et al*. (2019) [8]. The neem cake was found to be an excellent carrier material for dispersing the biocontrol agents. *T. viride* was found to have better shelf life 200 days and 35.7 x 10^7 CFU per gram and also inhibited the growth of pathogens under *in-vitro* conditions. The results of Bagwan (2010) [1] also quotes that the biocontrol organisms were found to be compatible with neem cake at different concentrations. The present findings were in correlation with the above findings.

Fig 1: Compatibility studies on different biocontrol agents with neem cake

Table 1: Compatibility studies on different biocontrol agents with neem cake

S. No	Treatments	Growth	Compatible/ Incompatible
T_1	*B. firmus* + Neem cake	++++	Compatible
T_2	*B. subtilis* + Neem cake	++++	Compatible
T_3	*P. lilacinum*+ Neem cake	++++	Compatible
T_4	*T. asperellum* + Neem cake	++++	Compatible
T_5	*B. firmus* alone	++++	
T_6	*B. subtilis* alone	++++	
T_7	*P. lilacinum* alone	++++	
T_8	*T. asperellum* alone	++++	

(- : No growth, ++ : Medium growth, ++++ : High growth).

Compatibility studies on different biocontrol agents

The results for compatibility studies between different biocontrol agents revealed that there was no inhibition zone observed among *B. firmus, B. subtilis, T. asperellum* (Figure 2). Nevertheless, *P. lilacinum* was found to be compatible with *T. asperellum* but showed incompatibility with *B. firmus* and *B. subtilis* (Figure 3, Table 2). It was found from the present study that *B. firmus* was compatible with *T. asperellum, B. subtilis*. Hence the *P. lilacinum* was not suitable for combined application with *B. firmus, B. subtilis, T. asperellum* in order to develop integrated nematode management strategy against *M. incognita*. This was in correlation with findings of Vijayashanthi (2020) [7]. The bacterial bioagents such as *B. firmus* and *B. subtilis* was compatible with each other and also with *T. asperullum*. The research findings of Latha *et al*. (2011) [6] states that *B. subtilis, Pseudomonas fluorescence and T. viride* was found to be compatible with each other with no inhibition zone.

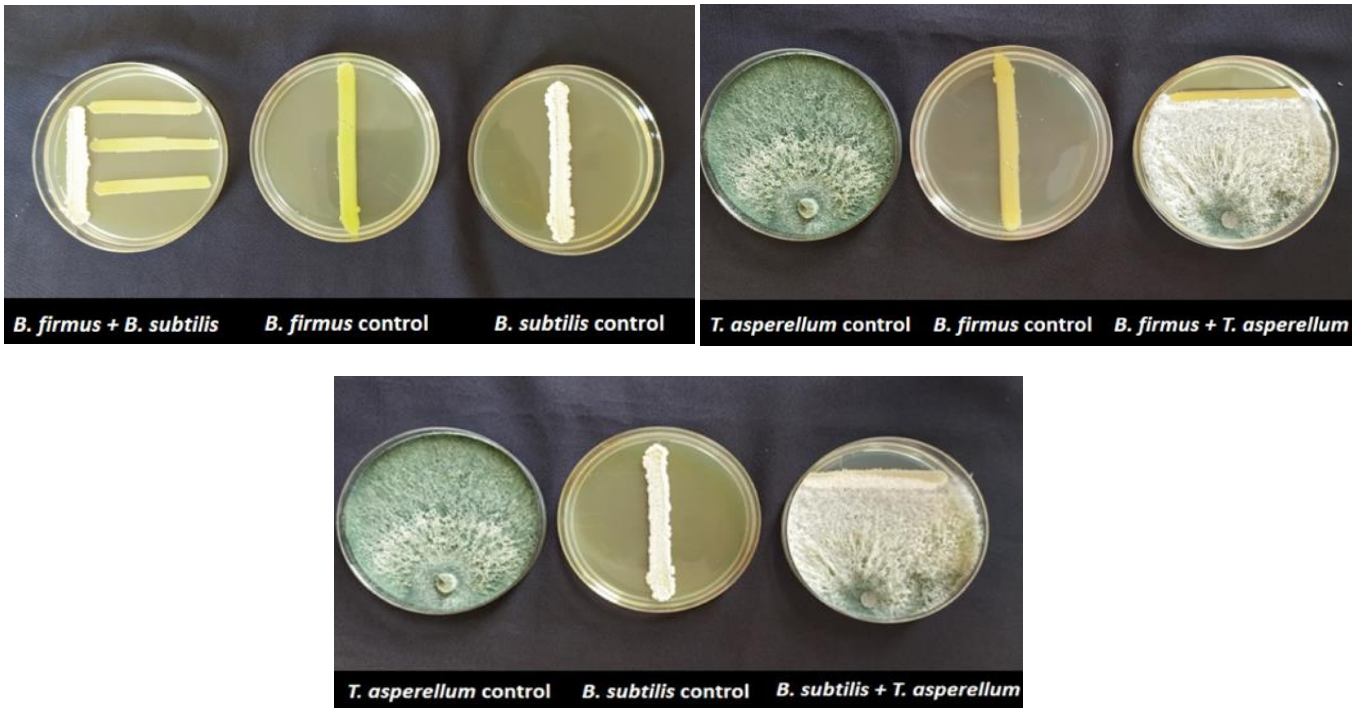

Fig 2: Compatibility studies between different biocontrol agents

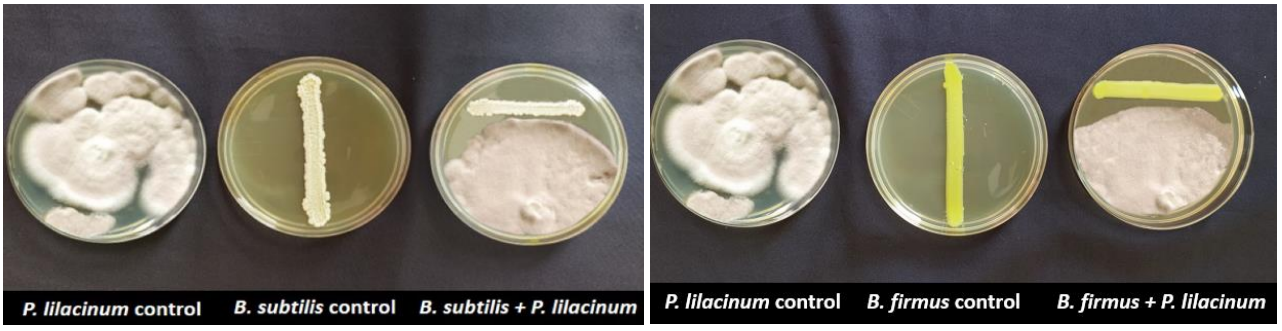

Fig 3: Incompatibility reaction between different biocontrol agents

Table 2: Compatibility studies on different biocontrol agents

S. No	Treatments	Growth	Compatible/ Incompatible
T_1	*B. firmus* + *B. subtilis*	+++	Compatible
T_2	*B. firmus* + *T. asperellum*	++++	Compatible
T_3	*B. firmus* + *P. lilacinum*	-	Incompatible
T_4	*B. subtilis* + *T. asperellum*	++++	Compatible
T_5	*B. subtilis* + *P. lilacinum*	-	Incompatible
T_6	*T. asperullum* +*P. lilacinum*	+++	Compatible
T_7	*B. firmus*	++++	
T_8	*B. subtilis*	++++	
T_9	*T. asperullum*	++++	
T_{10}	*P. lilacinum*	++++	

(- : No growth, ++ : Medium growth, ++++ : High growth)

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