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Biological control of *Heterodera cajani and* Fusarium udum *by* Bacillus subtilis, Bradyrhizobium japonicum *and* Glomus fasciculatum *on pigeonpea*

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Summary – *Bacillus subtilis, Bradyrhizobium japonicum* and *Glomus fasciculatum* were used alone and in combination for the management of a wilt disease complex of pigeonpea caused by the nematode *Heterodera cajani* and the fungus *Fusarium udum*. Application of all the three management agents alone or in combination to plants inoculated with the pathogens increased shoot dry weight, number of nodules, phosphorus content, and reduced nematode multiplication and wilting index. Application of *B. subtilis* alone to plants inoculated with either of the pathogens caused a similar increase in shoot dry weight as to that caused by *G. fasciculatum*. However, use of *B. subtilis* on plants inoculated with both the pathogens resulted in greater shoot dry weight than caused by *G. fasciculatum* or *B. japonicum*. Increase in shoot dry weight was greater when plants inoculated with pathogens were treated with *G. fasciculatum* plus *B. subtilis* or these two combined with *B. japonicum*. Application of all the three management agents against pathogens resulted in the greatest nodulation and the greatest reduction in nematode multiplication. Combined application of *G. fasciculatum* and *B. japonicum* increased root infection by *G. fasciculatum* while combined use with *B. subtilis* reduced mycorrhizal colonisation.

Résumé – ***Contrôle biologique d'*Heterodera cajani *et de* Fusarium udum *par* Bacillus subtilis, Bradyrhizobium japonicum *et* Glomus fasciculatum *sur pois d'Angole*** – *Bacilus subtilis, Bradyrhizobium japonicum* et *Glomus fasciculatum* sont utilisés, seuls ou en combinaison, pour le traitement d'un flétrissement complexe du pois d'Angole causé par le nématode *Heterodera cajani* et le champignon *Fusarium udum*. Les traitements à l'aide de ces trois agents, seuls ou en combinaison, effectués sur des plants inoculés par les deux parasites augmentent le poids sec des racines, le nombre de nodules et le taux de phosphore, et dimunuent le nombre de nématodes ainsi que l'indice de flétrissement. *B. subtilis* appliqué seul sur des plants inoculés par l'un ou l'autre parasite provoque un accroissement du poids sec des racines équivalent à celui causé par *G. fasciculatum*. Cependant, dans le cas de plants inoculés par les deux parasites, le poids sec des racines est supérieur à celui observé lors de l'utilisation de *G. fasciculatum* ou de *B. japonicum*. Cet accroissement du poids sec des racines est plus prononcé si le traitement comporte à la fois *G. fasciculatum* et *B. subtilis* ou si le troisième agent de contrôle, *B. japonicum*, est également présent. L'utilisation des trois agents de contrôle provoque une plus forte nodulation et une plus importante diminution du nombre des nématodes. Les combinaisons comportant *B. japonicum* et *G. fasciculatum* augmentent l'infestation racinaire par ce dernier alors qu'une combinaison de *G. fasciculatum* et de *B. subtilis* produit l'effet inverse.

Key-words : *Bacillus subtilis*, biological control, *Bradyrhizobium japonicum, Fusarium udum, Glomus fasciculatum, Heterodera cajani*, pigeonpea, wilt disease complex.

Pigeonpea, *Cajanus cajan* (L.) Millsp., is an important pulse crop of India and a major source of protein for most of the vegetarian population. Pigeonpea is susceptible to *Heterodera cajani* Koshy and *Fusarium udum* Butler. An extensive survey of cyst forming nematodes in Uttar Pradesh revealed that *H. cajani* is widely distributed (Husain *et al.*, 1989). Plants infected with *H. cajani* were stunted with marked chlorosis. *Fusarium udum* induced wilting and is destructive to the crop in certain states of northern India (Singh, 1983). Both pathogens together on pigeonpea cause a wilt disease complex which is a major constraint in the successful cultivation of this crop (Hasan, 1984; Siddiqui & Mahmood, 1995 *b*).

The microorganisms present in the rhizosphere may provide a defence for roots against pathogen attack. Of the various microorganisms present in the rhizosphere, vesicular-arbuscular mycorrhizal (VAM) fungi increase the plants' ability to absorb phosphorus, minor elements and water (Gerdemann, 1968; Hayman, 1982). They also limit yield losses due to pathogens by improving the phosphorus status of the host or by an antagonistic effect against the pathogens. Similarly, root-nodule bacteria fix atmospheric nitrogen and improve plant growth. The establishment of nodulating bacteria on or around the legume roots may also adversely affect establishment of some pathogens and reduce the damage they caused (Siddiqui & Husain, 1992; Ehteshamul-Haque & Gaf-

far, 1993). Some bacteria are also capable of providing substantial disease control against pathogens (Weller, 1988). For example, *Bacillus subtilis* Cohn *emend.* Prazmowski inhibited other pathogens and were effective in increasing yields of several crops (Weller, 1988; Siddiqui & Mahmood, 1993). *Bacillus subtilis* is not a nematode parasite but it has a high degree of larvicidal property (Siddiqui & Mahmood, 1995 *a*). It also produces some biologically active substances.

In the present study, an attempt was made to examine the role of *Bacillus subtilis, Bradyrhizobium japonicum* Jordan and *Glomus fasciculatum* (Thaxter *sensu* Gerd.) Gerdemann & Trappe alone or in combination for the management of a wilt disease complex of pigeonpea caused by *H. cajani* and *F. udum.*

Materials and methods

The pigeonpea cyst nematode *Heterodera cajani* and a wilt fungus *Fusarium udum* were used as test pathogens on pigeonpea, *Cajanus cajan* cv. UPAAS-120. A bacterium, *Bacillus subtilis,* a root nodule bacterium, *Bradyrhizobium japonicum,* and VAM fungus, *Glomus fasciculatum,* were used alone or in combination for the management of *H. cajani* and *F. udum.*

Plant culture

Seeds of pigeonpea cv. UPAAS-120 were surface sterilized by immersion in 0.1 % mercuric chloride for 2 min and washed three times in a sterile distilled water. The seeds were sown in 15 cm clay pots (two seeds/pot) containing 1 kg autoclaved sandy loam soil mixed with washed river sand and farm yard manure in the ratio of 3:1:1 (V/V) respectively. In the treatments where *G. fasciculatum* was inoculated, pots were filled with 950 g autoclaved soil; later, 50 g soil with VAM inoculum was added to make it 1 kg/pot. After germination, seedlings were thinned to one per pot. Pathogens were inoculated to the seedlings one week after germination. Inoculated plants were kept on a glass house bench at 25-27 °C. Pots were arranged in a randomised block design. The experiment was conducted twice, i.e., 1992 and 1993. The data presented in the paper were recorded in 1993. Pots were watered periodically and the experiment was terminated 90 days after inoculation.

Bacillus subtilis inoculum

Culture of *B. subtilis* was prepared on nutrient agar medium (Riker & Riker, 1936). Plates were incubated at 37 °C for 24 h, the bacteria were scraped from the plates, and a suspension prepared in distilled water to contain 10×10^8 bacteria cells/ml as determined by serial dilution plating procedure (Cappucinno & Sherman, 1983). One hundred ml of the bacterial suspension was poured into 100 g autoclaved soil and 100 seeds were mixed in the soil. Seeds were dried for 1 h at room temperature. Thus each seed contains approximately 10×10^8 bacterial cells.

Bradyrhizobium japonicum inoculum

One hundred g commercial culture of *B. japonicum* (pigeonpea strain) was suspended in 1000 ml distilled water and 10 ml (equivalent to 1 g inoculum) was added around the seeds of each pot at the time of sowing.

Glomus fasciculatum inoculum

The air dried *G. fasciculatum* inoculum was obtained from a culture center of Bangalore, India. Inoculum of *G. fasciculatum* was prepared on *Chloris gayana* (Rhodes grass) grown in sandy loam soil mixed with washed river sand and farm yard manure in a ratio of 3:1:1 (V/V), respectively. The population of *G. fasciculatum* in the inoculum was assessed by most probable number method (Porter, 1979). Fifty g inoculum with soil was added around the seeds to inoculate 500 infecting propagules of *G. fasciculatum* per pot (1 g inoculum contains ten infective propagules). The crude inoculum consists of soil, extra matrical spores and sporecarps, hyphal fragments and infected Rhodes grass segments.

Heterodera cajani inoculum

Heterodera cajani was collected from a pigeonpea field and multiplied using J2 from a single cyst. The cysts from this population were later identified using cone top and juvenile characters as described by Koshy *et al.* (1971). The cysts were collected from the roots and placed for hatching in root exudates. 500 freshly hatched J2 were used as inoculum for each plant.

Fusarium udum inoculum

Fusarium udum was isolated from infected pigeonpea roots and maintained on potato dextrose agar (PDA). Inoculum of the fungus was prepared in Richards liquid medium for 15 days at 25 ± 2°C (Riker & Riker, 1936). Mycelium was collected on blotting sheets and excess water and nutrients were removed by pressing it between two folds of blotting sheets. The inoculum, in the form of mycelium suspension, was prepared by mixing 10 g of mycelium in 100 ml sterilised water and blending for 30 s in a Waring blender. 10 ml of this suspension contained 1 g of mycelium.

Inoculation technique

For inoculation of the pathogens, soil around the roots was carefully removed and a suspension of inoculum was poured around the roots uniformly. Water was poured on the controls in the same way. There were eight treatments :

1 : control,
2 : *B. japonicum* (BJ),
3 : *G. fasciculatum* (GF),
4 : *B. subtilis* (BS),

Table 1. *Overall effect of biocontrol agents on the growth of pathogen inoculated and non-inoculated plants (pooled data).*

Treatments	Plant length (cm)	Shoot dry weight (g)	No. of nodules per root system	Percent root infection by *G. fasciculatum*	Isolation of *B. subtilis* in percent from		Nematode population		Phosphorus contents in mg per g leaf dry wt.	Wilting index
					Females/ cyst	Eggs	Females/ cysts per root system + 1 kg soil	J2 in 1 kg soil		
Control	173.7	8.42	51	62	-	-	-	-	3.816	-
H. cajani	150.5	7.30	34	56	6	6	24	3130	3.369	-
F. udum	143.8	6.89	30	48	-	-	-	-	3.211	1.6
H. cajani + *F. udum*	97.8	4.98	18	37	5	4	21	2788	3.109	2.9
C. D. $P \leq 0.05$	1.2	0.06	2.4	2.2	3	4	2	59	0.069	-

5 : BJ + GF,
6 : BJ + BS,
7 : GF + BS,
8 : BJ + GF + BS.

Each of these eight treatments was tested with three pathogen treatments which were *H. cajani, F. udum* and *H. cajani* plus *F. udum*. A control not treated with pathogens was included with each of the eight treatments. So, in total, there were thirty two treatments and each was replicated five times.

Observations

Data were recorded on plant height, shoot dry weight, number of nodules, percentage of root infection by *G. fasciculatum,* phosphorus content of shoots, number of cysts and larvae in the soil. The nematode population from soil was extracted by Cobb's sieving and decanting technique followed by Baermann funnel (Southey, 1986) while cysts were extracted by Fenwick can. Females on the roots were counted by staining the roots in cotton blue before examination under the stereomicroscope. The proportion of root colonized by *G. fasciculatum* was determined by the grid line intersecting method (Giovannetti & Mosse, 1980) after clearing roots with KOH (Phillips & Hayman, 1970) and staining the roots in 0.05 % trypan blue-lactophenol. Phosphorus content of the shoots were determined by the molybdate blue method (Murphy & Riley, 1962) after dry ashing. *Bacillus subtilis* was re-isolated from eggs and females/cysts of *H. cajani* to determine the infection of *B. subtilis* on nematode population. For re-isolation, 20 eggs and the same number of cysts/females were surface sterilized with 0.1 % mercuric chloride for 2 min, washed three times in distilled water and placed in nutrient agar medium for bacterial growth. The plates were incubated as described earlier. Bacterial growth, if found, was identified. A wilting index was determined by scoring the disease severity on a scale ranging from 0 (no wilting) to 5 (severe wilting). All the data collected were analysed statistically using single factor analysis, and critical differences (C.D.) were calculated at $P \leq 0.05$.

Results

Overall effect of biocontrol agents on pathogen inoculated and non-inoculated plants

Plant length, shoot dry weight, number of nodules, phosphorus content and VAM colonisation on roots was greater in plants without pathogens (control) compared to pathogen inoculated plants (Table 1). Plant length, shoot dry weight, number of nodules, phosphorus content, and VAM colonisation on roots were considerably reduced when plants were inoculated with *H. cajani* or *F. udum,* but damage caused by *F. udum* was greater than by *H. cajani*. The greatest reduction in plant length, shoot dry weight, number of nodules, phosphorus content and VAM colonisation on roots was observed when both pathogens were inoculated together. Multiplication of *H. cajani* was less in the presence of *F. udum* than when *H. cajani* was inoculated alone.

Effect of biocontrol agents on plants without test pathogens

Inoculation of *G. fasciculatum* to plants without pathogens resulted in greater shoot dry weight than when plants were treated with *B. japonicum* or *B. subtilis* (Table 2). Shoot dry weight of plants without pathogens and inoculated with *B. japonicum* was higher than when inoculated with *B. subtilis*. Greatest shoot dry weight was observed when plants without pathogens were treated simultaneously with *B. japonicum, G. fasciculatum* and *B. subtilis* or with *G. fasciculatum* and *B. japonicum*.

Only a few nodules were observed on the roots where *B. japonicum* was not inoculated (Table 2). Nodulation was found to be increased where *B. japonicum* was used with *G. fasciculatum*. Percent root colonisation by VAM

Table 2. *Effect of biocontrol agents on the growth of non pathogen inoculated plants.*

Treatments	Plant length (cm)	Shoot dry weight (g)	No. of nodules per root system	Percent root infection by *G. fasciculatum*	Phosphorus contents in mg per g leaf dry weight
Control	154.6	7.47	10	–	3.214
B. japonicum (BJ)	172.2	8.12	87	–	3.410
G. fasciculatum (GF)	176.8	8.65	12	62	4.160
B. subtilis (BS)	162.9	7.86	9	–	3.324
BJ + GF	182.8	8.92	95	65	4.234
BJ + BS	175.2	8.46	85	–	3.450
GF + BS	179.6	8.80	15	60	4.314
BJ + GF + BS	185.4	9.06	97	61	4.425
C.D. $P \leq 0.05$	6.1	0.25	6.7	3.7	0.241

was not influenced by *B. japonicum* and *B. subtilis* in plants without pathogens. Inoculation of *G. fasciculatum* alone or in combination with *B. subtilis* and *B. japonicum* to plants without pathogens resulted in an increase of the phosphorus content. However, no increase in phosphorus was observed when *B. subtilis* or *B. japonicum* was used alone against non-pathogen inoculated plants.

Effect of biocontrol agents on plants inoculated with *H. cajani*

Treatment of all the three biocontrol agents, i.e., *B. japonicum*, *B. subtilis* and *G. fasciculatum* individually increased shoot dry weight of *H. cajani* inoculated plants (Table 3). *B. subtilis* caused greater increase in shoot dry weight than *B. japonicum*. However, *G. fasciculatum* caused the same shoot dry weight increase that caused by *B. japonicum*. Treatment of all the three biocontrol agents together or *B. subtilis* with *G. fasciculatum* resulted in the greatest shoot dry weight increase in *H. cajani* inoculated plants.

Inoculation of *H. cajani* suppressed nodulation as compared to control while treatment of *G. fasciculatum* or *B. subtilis* or both to *B. japonicum* plus *H. cajani* inoculated plants resulted in increased nodulation (Table 3). Root colonisation by VAM and phosphorus contents were found to be reduced in the presence of *H. cajani* compared to plants without *H. cajani*. Application of *B. japonicum* or *B. subtilis* or both resulted in increased phosphorus content. However, maximum phosphorus content were observed when *G. fasciculatum* was used with either or both the biocontrol agents on plants inoculated with *H. cajani*.

The results of re-isolation of *B. subtilis* from eggs and females/cysts were not significant (Table 3). *B. subtilis* caused higher reduction in nematode population than *G. fasciculatum* or *B. japonicum*. Application of all three biocontrol agents together resulted in the highest reduction in nematode population.

Effect of biocontrol agents on plants inoculated with *F. udum*

The addition of *G. fasciculatum* to plants inoculated with *F. udum* caused an increase in shoot dry weight similar to that caused by *B. subtilis* (Table 4). However, the addition of *B. japonicum* to *F. udum* inoculated plants caused less increase in shoot dry weight compared to *B. subtilis* or *G. fasciculatum*. Use of all three biocontrol agents together on *F. udum* inoculated plants resulted in a greater shoot dry weight than the combined use of any of these biocontrol agents.

Only a few nodules were observed where *B. japonicum* was not inoculated (Table 4). Nodulation was the same when *B. japonicum* alone or *B. japonicum* plus *B. subtilis* were added to *F. udum* inoculated plants. Nodulation was found to be increased when *B. japonicum* was inoculated with *G. fasciculatum* compared to plants with *B. japonicum* alone. Maximum number of nodules were observed when all three biocontrol agents were inoculated. Root colonisation by VAM was reduced in the presence of *Bacillus subtilis* while it was increased in the presence of *B. japonicum*. Treatment of *B. subtilis* or *B. japonicum* had no effect on phosphorus content. However, application of *G. fasciculatum* alone or with *B. subtilis* and *B. japonicum* increased phosphorus content of *F. udum* inoculated plants. Wilting index was equal to three when *F. udum* was inoculated alone. Addition of anyone of the biocontrol agents to *F. udum* inoculated plants reduced the wilting index to two. Combined application of biocontrol agents reduced the wilting index to only one.

Table 3. *Effect of biocontrol agents on the growth of* Heterodera cajani *inoculated plants.*

Treatments	Plant length (cm)	Shoot dry weight (g)	No. of nodules per root system	Percent root infection by *G. fasciculatum*	Isolation of *B. subtilis* in percent from		Nematode population		Phosphorus contents in mg per g leaf dry wt.
					Females	Eggs	Females/ cysts per root system per kg soil	J2 population per kg soil	
Control	115.6	5.82	5	–	–	–	48	5740	2.645
B. japonicum (BJ)	143.2	6.95	59	–	–	–	37	4460	2.890
G. fasciculatum (GF)	149.6	7.15	4	57	–	–	30	3.480	3.610
B. subtilis (BS)	153.4	7.30	6	–	7	5	24	2970	2.980
BJ + GF	156.8	7.52	65	62	–	–	20	2540	3.880
BJ + BS	158.2	7.71	58	–	8	6	15	2320	3.040
GF + BS	161.3	7.89	5	53	4	6	12	1890	3.940
BJ + GF + BS	165.8	8.02	70	51	5	7	9	1640	3.970
C.D. $P \leq 0.05$	5.4	0.21	4.4	2.9	6	5	4	95	0.178

Table 4. *Effect of biocontrol agents on the growth of* Fusarium udum *inoculated plants.*

Treatments	Plant length (cm)	Shoot dry weight (g)	No. of nodules per root system	Percent root infection by *G. fasciculatum*	Phosphorus contents in mg per g leaf weight	Wilting index
Control	112.4	5.28	4	–	2.530	3
B. japonicum (BJ)	138.6	6.60	51	–	2.760	2
G. fasciculatum (GF)	143.7	6.98	7	49	3.440	2
B. subtilis (BS)	144.9	7.03	3	–	2.810	2
BJ + GF	148.4	7.18	58	54	3.570	1
BJ + BS	147.9	7.10	50	–	2.910	1
GF + BS	154.7	7.35	5	43	3.710	1
BJ + GF + BS	160.1	7.56	64	45	3.960	1
C.D. $P \leq 0.05$	4.7	0.21	4.6	4.3	0.210	–

EFFECT OF BIOCONTROL AGENTS TO PLANTS INOCULATED WITH *H. CAJANI* PLUS *F. UDUM*

Addition of *B. subtilis* to plants inoculated with both pathogens caused similar increase in shoot dry weight than that caused by *G. fasciculatum* (Table 5). Greater increase in shoot dry weight of plants inoculated with the pathogens was observed when treated with *G. fasciculatum* than with *B. japonicum*. Use of *G. fasciculatum* plus *B. subtilis* resulted in greater shoot dry weight than use of *B. japonicum* plus *G. fasciculatum* or *B. japonicum* plus *B. subtilis*. Greatest dry shoot weight was observed when all the three biocontrol agents were used together.

Greater number of nodules were observed when plants inoculated with both pathogens were treated with *B. japonicum* plus *G. fasciculatum* or these two plus *B. subtilis* compared to plants inoculated only with the pathogens (Table 5). Root colonisation by VAM increa-

Table 5. *Effect of biocontrol agents on the growth of* Heterodera cajani *plus* Fusarium udum *inoculated plants.*

Treatments	Plant length (cm)	Shoot dry weight (g)	No. of nodules per root system	Percent root infection by *G. fasciculatum*	Isolation of *B. subtilis* in percent from		Nematode population		Phosphorus contents in mg per g leaf dry wt.	Wilting index
					Females/ cyst	Eggs	Females/ cysts per root system + 1 kg soil	J2 in 1 kg soil		
Control	60.4	3.07	6	–	–	–	41	4810	2.410	5
B. japonicum (BJ)	76.2	4.40	24	–	–	–	32	3960	2.680	4
G. fasciculatum (GF)	86.6	4.63	7	38	–	–	26	3470	3.210	3
B. subtilis (BS)	94.8	4.89	5	–	7	4	21	2860	2.760	3
BJ + GF	106.9	5.10	30	44	–	–	17	2390	3.560	2
BJ + BS	110.8	5.57	26	–	5	6	13	2040	2.840	2
GF + BS	116.9	5.86	6	34	4	3	9	1560	3.670	2
BJ + GF + BS	129.6	6.28	39	32	5	2	6	1210	3.740	2
C.D. $P \leq 0.05$	5.2	0.23	2.9	2.5	5	4	3	77	0.127	–

sed in the presence of *B. japonicum,* while *B. subtilis* had adverse effect on VAM colonisation. Inoculation of *G. fasciculatum* increased phosphorus content of the plants. Maximum phosphorus content were observed when *G. fasciculatum* was used with *B. japonicum,* with *B. subtilis,* or with both. *B. subtilis* caused higher reduction in nematode multiplication than *G. fasciculatum.* Highest reduction in nematode multiplication was observed when all three biocontrol agents were used together. Both pathogens together resulted in the wilting index of five. Use of two or three biocontrol agents reduced the wilting index to only two.

Discussion

Bacillus subtilis reduced multiplication of pigeonpea cyst nematode *H. cajani,* resulting in improved plant growth. Treatment with *B. subtilis* also reduced wilting index of *F. udum* inoculated plants. Improvement in plant growth can be attributed to inhibitory effects of *B. subtilis* against pathogens (Yuen *et al.,* 1988; Siddiqui & Mahmood, 1993, 1995 *a*). Previous studies indicated that treatment of *B. subtilis* increased the yield of several crops (Merriman *et al.,* 1974; Turner & Backman, 1986). Additionally, *B. subtilis* improved plant growth by inhibiting non-parasitic root pathogens, producing biologically active substances or by transforming unavailable minerals and organic compounds into forms available to plants (Broadbent *et al.,* 1977). Moreover, a noncellular extract of *B. subtilis* was also reported to have a high degree of larvicidal properties to *H. cajani* (Gokte & Swarup, 1988).

Treatment with *G. fasciculatum* improved the growth of nematode inoculated plants by reducing the multiplication of the pathogens as reported by Bagyaraj *et al.* (1979). The wilting index of *F. udum* inoculated plants was also reduced by *G. fasciculatum.* Krishna and Bagyaraj (1983) reported that *G. fasciculatum* reduced the severity of disease caused by *Sclerotium rolfsii* while Dehne and Shonbeck (1975) observed that *Glomus mosseae* reduced *Fusarium* wilt of tomato. Reduced damage by pathogens in mycorrhizal plants may be due to physiological and biochemical changes in the host or to an increase in the flow of nutrients which gives mechanical strength (Schonbeck, 1979). In addition, inoculation of *G. fasciculatum* resulted in increase in phosphorus content which offsets symptoms of the nematode infestation (Hussey & Roncadori, 1982). Treatment with *G. fasciculatum* is also reported to increase phenylalanine and serine in tomato roots (Suresh, 1980) and these aminoacids have an inhibitory effect on nematodes (Reddy, 1974).

Treatment with *B. japonicum* also resulted in reduced damage as reported earlier (Siddiqui & Husain, 1992; Ehteshamul-Haque & Gaffar, 1993). *Bradyrhizobium* is reported to produce antipathogenic substances and to reduce namatode multiplication (Drapeau *et al.,* 1973; Siddiqui & Husain, 1992; Siddiqui & Mahmood, 1994). Combined application of *B. japonicum* with *G. fasciculatum* resulted in more nodulation and greater phosphorus content. This provided better plant growth as reported by Manjunath and Bagyaraj (1984). Use of *G. fasciculatum* and *B. subtilis* was more beneficial in reducing damage caused by pathogens than individual inoculations. This was probably due to positive interaction of both organisms. *B. subtilis* has an inhibitory effect on pathogens and *G. fasciculatum* increases plants ability

to absorb phosphorus, minor-elements and water (Hayman, 1982), besides, increasing plant resistance.

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