

**EFFICACY OF DIFFERENT DAYS OLD LIQUID FORMULATION OF *BACILLUS SUBTILIS*, BG42 ON HATCHING AND MORTALITY OF ROOT KNOT NEMATODE, *MELOIDOGYNE INCOGNITA***

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Gerbera or Transvaal daisy (*Gerbera jamesonii* Hook) is one of 10 most important cut flowers in the world and according to the global trends in floriculture, it occupies the fourth place among cut flowers. It is in considerable demand in both domestic and export markets. Of the several limiting factors that cause serious concern to commercial production of gerbera, plant parasitic nematodes are most important. Root knot nematodes belonging to *Meloidogyne* spp. are predominant in India. In India, yield losses due to *Meloidogyne incognita* in gerbera were estimated to the tune of 31.1 %. Now-a-days the use of bioagents are considered as one of the alternative approaches for the management of nematodes as it is safe to environment, no health hazards to human beings, economic and easily available to farmers in comparison to chemicals. Among the plant growth promoting rhizobacteria (PGPR), *Pseudomonas* and *Bacillus* are the genera most commonly described as having PGPR (Barea *et al.*, 2005). Many *Bacillus* spp. are known to suppress the nematodes, particularly *M. incognita*. *B. subtilis*, is an adept rhizobacterium and has gained global attention as a biopesticide for the control of several plant diseases. It is known to inhibit a number of soil borne pathogens (Edgecomb and Manker, 2006). The potential of this biocontrol bacterium has been reported to be effective against plant pathogenic nematodes (Siddiqui and Ehteshamul, 2001) and other soil borne pathogens.

The present investigation was carried out to evaluate the efficacy of different days old liquid formulation of *B. subtilis*, BG42 on hatching and mortality of *M. incognita* in gerbera. Ten isolates of *Bacillus* spp. were isolated from gerbera rhizosphere from Tamil Nadu districts namely Coimbatore, The Nilgiris, Salem and Krishnagiri. Bioefficacy of *Bacillus* isolates was assayed against root knot nematode by hatching and mortality tests. Among the ten isolates screened, the highest inhibition in egg hatching and highest per cent mortality of *M. incognita*

juveniles was observed at *Bacillus* isolate BG42. The partial 16S rDNA sequences of the isolated strain BG42 showed 99% identity with *Bacillus subtilis* and were deposited in GenBank under accession numbers of KM588210. The most promising *B. subtilis* strain BG 42 from the above tests were selected to study their efficiency of different days old liquid formulation against *M. incognita in vitro*. For preparation of cell free filtrate, a single colony of *B. subtilis* strain BG 42 was cultured in flask containing Nutrient broth and incubated at  $28 \pm 2^\circ\text{C}$  on a shaker at 100 rpm for 2-3 days.

The culture was subsequently passed through sterilized Whatmann filter paper No.1 and 42 respectively, concentrated by centrifugation at 6000 rpm for 10 min and the supernatant was collected and finally passed through a Millipore filter of  $0.02\mu\text{m}$ . A replicated trial was conducted to study the effect of different days old cell free filtrates of *B. subtilis* strain BG 42 on hatching of *M. incognita in vitro*. Ten ml each of cell free culture filtrate concentrations (5, 15 and 25%) were taken in a syracuse dish and Nutrient broth as a standard check and distilled water without bacteria served as a control. One egg mass of root knot nematode was placed in each plate and kept at  $28 \pm 2^\circ\text{C}$ . The experiment was replicated 4 times. Observation on number of hatched juveniles was made after 24, 48 and 72 h of exposure at 3, 30, 75, 120, 165 and 210 days old cultures. About ten ml of various days old cell free filtrates of *B. subtilis* strain BG 42 of different concentrations (5, 15 and 25%) were poured into separate syracuse dish. The second stage juveniles of *M. incognita* were introduced into each dish @ 100 juveniles in 0.1 ml of sterile water and incubated at  $28 \pm 2^\circ\text{C}$ . Nutrient broth without bacteria served as a standard check along with distilled water as a control. Each treatment was replicated 4 times. Observations were recorded on the mortality of juveniles after 24, 48 and 72 hours of exposure period at 3, 30, 75, 120, 165 and 210 days old cultures.

Table 1: Effect of different days old *B. subtilis* strain BG 42 culture filtrate on percent hatching of *M. incognita* eggs

Days	Concentration of <i>B. subtilis</i> strain BG 42								
	5%			15%			25%		
	24	48	72	24	48	72	24	48	72
3	39.00 (38.65)	56.67 (48.83)	88.67 (70.33)	22.67 (28.43)	36.67 (37.27)	37.67 (37.86)	04.33 (12.01)	06.00 (14.18)	07.00 (15.34)
30	40.33 (39.43)	59.33 (50.38)	89.67 (71.25)	24.00 (29.33)	37.00 (37.46)	38.00 (38.06)	05.00 (12.92)	06.67 (14.96)	08.00 (16.43)
75	42.00 (40.40)	62.33 (52.14)	90.67 (72.21)	25.00 (30.00)	37.67 (37.86)	39.33 (38.84)	06.67 (14.96)	07.33 (15.71)	08.33 (16.78)
120	44.00 (41.55)	66.00 (54.33)	92.00 (73.57)	28.00 (31.95)	38.67 (38.45)	40.00 (39.23)	07.00 (15.34)	07.67 (16.07)	08.67 (17.12)
165	45.00 (42.13)	68.67 (55.96)	92.67 (74.29)	30.33 (33.42)	39.00 (38.65)	40.67 (39.62)	07.33 (15.71)	08.33 (16.78)	10.00 (18.43)
210	48.67 (44.24)	75.00 (60.00)	93.33 (75.04)	31.00 (33.83)	39.33 (38.84)	41.00 (39.82)	08.67 (17.12)	09.33 (17.79)	10.67 (19.06)
Nutrient broth	33.67 (35.47)	67.33 (55.14)	98.00 (81.87)	33.67 (35.47)	67.33 (55.14)	98.00 (81.87)	33.67 (35.47)	67.33 (55.14)	98.00 (81.87)
Distilled water	34.33 (35.87)	67.67 (55.35)	98.33 (82.58)	34.33 (35.87)	67.67 (55.35)	98.33 (82.58)	34.33 (35.87)	67.67 (55.35)	98.33 (82.58)
Mean	40.88 (39.72)	65.38 (54.02)	92.92 (75.14)	27.81 (27.80)	42.24 (35.46)	47.81 (39.41)	10.38 (15.44)	16.10 (18.83)	21.52 (23.13)
C D ( $p=0.05$ ) T-11.08, H-6.78, C-6.78, TxH19.19, HxC-11.75, TxC-19.19, TxCxH=33.24									

T=Treatment, H=Exposure hours, C=Concentration, Figures in rectangular parentheses are square root transformed values

Hatching study revealed a negative relationship between the concentration of the liquid formulation and the number of eggs hatched. Significant reduction in egg hatching was observed with different days old cultures of *B. subtilis* strain BG 42 with the least being observed with the three day old culture of BG42 (7.00%) at 25% concentration after 72h exposure period (Table 1). Similar trend was

also observed at 15 and 5 % conc. The highest egg hatching was observed with the culture filtrate of 210 days old *B. subtilis* strain BG 42 at 5% conc. at an exposure period of 72h. The report of the inhibition in egg hatching of rice root nematode due to the production of secondary metabolites stated as one of the mechanism of *Bacillus* spp. by Padgham and Sikora (2007) is coinciding with the results of the present study.

Table 2: Effect of different days old *B. subtilis* strain BG 42 culture filtrate on percent mortality of *M. incognita* juveniles

Days	Concentration <i>B. subtilis</i> strain BG 42								
	5%			15%			25%		
	24	48	72	24	48	72	24	48	72
3	05.00 (12.92)	12.00 (20.27)	12.67 (20.85)	18.33 (25.35)	35.33 (36.47)	36.33 (37.07)	48.00 (43.85)	86.00 (68.03)	87.00 (68.87)
30	04.67 (12.48)	11.00 (19.37)	11.33 (19.67)	15.33 (23.05)	32.67 (34.86)	33.67 (35.47)	47.33 (43.74)	84.33 (66.68)	86.00 (68.03)
75	04.33 (12.01)	10.33 (18.75)	11.33 (19.67)	15.00 (22.79)	30.67 (33.63)	31.67 (34.24)	45.33 (45.32)	79.00 (62.73)	80.00 (63.43)
120	04.00 (11.54)	07.67 (16.07)	08.67 (17.12)	13.33 (21.42)	28.67 (32.37)	30.00 (33.21)	42.00 (40.40)	75.00 (60.00)	76.33 (60.89)
165	02.67 (9.40)	07.00 (15.34)	08.00 (16.43)	12.33 (20.56)	26.00 (30.66)	27.33 (31.52)	41.33 (40.01)	69.33 (56.37)	71.00 (57.42)
210	02.00 (8.13)	05.67 (13.77)	07.00 (15.34)	10.00 (18.43)	24.67 (29.78)	25.67 (30.44)	40.67 (39.62)	66.33 (54.53)	68.67 (55.96)
Nutrient broth	00.67 (4.68)	01.67 (7.42)	02.00 (8.13)	00.67 (4.68)	01.67 (7.42)	02.00 (8.13)	00.67 (4.68)	01.67 (7.42)	02.00 (8.13)
Distilled water	00.67 (4.68)	01.33 (6.63)	01.67 (7.42)	00.67 (4.68)	01.33 (6.63)	01.67 (7.42)	00.67 (4.68)	01.33 (6.63)	01.67 (7.42)
Mean	03.00 (9.48)	07.08 (14.70)	07.83 (15.58)	12.14 (17.04)	25.67 (25.65)	26.67 (26.26)	37.90 (31.79)	65.95 (46.97)	67.29 (47.84)
CD=( $p=0.05$ ) T-5.90, H-3.61, C-3.61, TxH-10.23, HxC-6.26, TxC-10.23, TxCxH=17.72									

T=Treatment, H=Exposure hours, C=Concentration, Figures in rectangular parentheses are arc sine transformed values

This study revealed that the increase in concentration of the culture filtrate resulted in decrease in egg hatching. Maximum egg hatching of 98.33% was recorded in distilled water followed by the NA broth with 98.00% at 72h of exposure. The study conducted by Ann (2013) also indicated the effectiveness of *Bacillus* strains MPB04 and MPB93 against *M. incognita* due to its strong proteolytic activity. The biocidal property of the culture filtrate increased with increase in exposure period and increase in concentration.

Gradual increase in the juvenile mortality was observed with increase in concentration of culture filtrate. Significant differences in the juvenile mortality were noticed in the culture of different days old *B. subtilis* strain BG 42 at all the exposure period viz., 24, 48 and 72h respectively. The highest juvenile mortality of 87.00% was recorded in the culture filtrate of BG42 at 25% concentration after 72h exposure

(Table 2). The Lowest juvenile mortality was recorded in 210 days old culture filtrate of *B. subtilis* strain BG 42 (7%) at 5% conc. The present investigation is in accordance with the study conducted by Pankaj *et al.* (2010), Mohammed *et al.* (2007), Ramezani Moghaddam *et al.* (2013), Huang *et al.* (2009) and Al-Banna and Horani (2004) also proved the biocontrol potential of *B. thuringiensis*, *B. megaterium*, *B. pumilus* over eggs and juveniles of different species of *Meloidogyne* viz., *M. incognita*, *M. javanica* and *M. graminicola*.

It could be concluded that, in the hatching studies, 25% concentration of 3 day old formulation recorded minimum egg hatching of 7 juveniles/egg mass after 72h of exposure. Similarly, the 3 day old culture at 25 % concentration recorded maximum juvenile mortality of 87%. Antagonist effect was found to decrease with increase in age of the culture.

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