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# Reaction of Bt Cotton to *Rotylenchulus reniformis* Infection

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**Experiments were conducted twice to evaluate the influence of Bt cotton on *Rotylenchulus reniformis* under laboratory (28$^o$± 2$^o$C) and glasshouse conditions. Three Bt hybrids *viz.,* Bunny Bt, RasiBt and Jagannath fusion Bt were compared with their respective non Bt (Refugia) for their impact on egg hatching, juveniles attraction, root penetration and development of *R. reniformis.* Jagannath fusion Bt and Rasi Bt were effective in inhibiting/delaying the egg hatching as compared to their non Bt lines whileBunny Bt had no significant effect in inhibiting the egg hatching over its non Bt. But Bt hybrids had no significant influence in causing mortality of juveniles, attraction of infective pre adult ($J_4$) and penetration of *R. reniformis*. In all Bt hybrids, on 7$^{th}$ day after inoculation reduction in number of females with egg mass was observed over its non Bt. The penetrated juveniles of *R. reniformis* were not able to become adults in Bt cotton.**

**Key words:** Bt hybrids, Bunny Bt, Rasi Bt, Jagannath fusion Bt, Non Bt and *Rotylenchulus reniformis*

*Bacillus thuringiensis*has has been used as biological control agent against various insect pests for the past one century. Due to increasing importance of cotton crop and magnitude of yield loss due to nematodes Genetically modified (GM) crop with *Bacillus thuringiensis* (Bt) is considered as an alternative technology to manage nematode problem as it was successfully proved in tomato, potato, arabidopsis, alfalfa etc. (Urwin *et al.,* 1998; Urwin *et al.,* 2001; Samac and Smigockis, 2003; Li *et al.,*2007*).*

So far Bt cotton originally evolved for the management of boll worms has not been studied for their effectiveness against reniform nematode, *Rotylenchulus reniformis*, a key nematode pest of cotton. Therefore, the influence of three Bt cotton hybrids on reniform nematode was studied under laboratory and greenhouse conditions.

## Materials and Methods

### *Pure culturing of reniform nematode*

Reniform nematode, required for the study was collected from single egg mass of *R. reniformis* from papaya roots. The egg mass was allowed to hatch in tap water for 15 days and the juveniles were used for monoculturing of nematodes on castor and used for the present study (Sivakumar and Seshadri, 1971).

### *Collection of root exudates of Bt and non Bt cotton*

Three Bt cotton seeds *viz*., Bunny, Rasi and Jagannath fusion and their respective non Bt (Refugia) were sown in earthen pots (1 kg) and on germination thinned to 5 plants/ pot. Plants were maintained under glasshouse conditions at 30 ± 2ºC. After 50 days the plants were kept unwatered for two days. On the third day 500 ml water was poured to each of the plants and the root exudates were collected in a pan placed underneath the pots and filtered through a Waatmann No 1 filter paper and stored in a conical flask under room temperature (30± 2ºC) and used for the present study.

### *Hatching and mortality tests*

Five ml of root exudates collected from each Bt and non Bt hybrids were transferred to a 6 cm diameter Petri dish. Uniform sized five egg masses of *R. reniformis* were placed in each Petri dish and incubated under room temperature. The number of hatched out juveniles was recorded for 7 days at 24 h interval after exposure to the root exudates.While for the mortality study $J_4$ (pre adult stage) were used @ 100 per Petri dish (6 cm diameter) and the per cent mortality of $J_4$ of *R. reniformis* was recorded at 24 h interval for a week.

### *Attraction test*

Bt and non Bt cotton seedlings were raised by roll towel method and cut at the growing end at third day after sowing. Root bits were surface sterilized using 70 per cent ethanol for one minute and washed with distilled water and used for the attraction study as detailed below.

The effect of all the three Bt and non Bt root exudates on the attraction of *R. reniformis* juveniles *in vitro* was studied by agar plate method (Azmi and Jairajpuri, 1976) with slight modification.Molten

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water agar (2 per cent) was poured in to Petri dishes and kept in an incubator at 27º C for 24 h. Five circles with equal distance from the centre point of Petri dish were marked on the bottom of Petri dish to denote 20, 40, 60, 80 and 100 per cent area of attraction. Surface sterilized root bits (1 cm length) were placed at the centre of each Petri dish. The pre adult ($J_4$) stage of *R. reniformis* were introduced in each Petri dish @ 100 nematodes/ Petri dish near the periphery of outer rim in equal numbers at equidistance in four different directions and maintained at room temperature. The number of nematodes in the Petri dishes in different circles was counted at 24 h interval for 7 days and the per cent attraction of test Bt and non Bt was computed.

***Penetration test***

An experiment was conducted under glass house conditions at 30± 2ºC to study root penetration by *R. reniformis* in Bt and non Bt cotton. The Bt and non Bt hybrids were raised in earthen pots (1 kg) containing steam sterilized pot mixture. In one month old plants, the pre adult stage of juveniles were inoculated @ 1000 pot near the root zone. One plant was uprooted every day from the time of inoculation to study the penetration, development and fecundity for 10 days. Root portion of uprooted plant was washed gently under tap water, cut in to small bits of 1 cm length with sharp razor, immersed in boiled lactophenol- acid fuchsin, washed in water, destained with clear lactophenol and examined under a microscope to study penetration (McBeth *et al.,* 1941).

The above experiments were carried out with three Bt varieties *viz*, Bunny Bt (BGI – *Cry* 1Ac gene), Rasi Bt (BGII- *Cry* 1Ac and *Cry* 2Ab) and Jagannath Bt (Fusion- *Cry* 1Ab and *Cry* 1Ac) with the concerned refugia seeds as non Bt using paired 't' test.Thirteen replications were maintained for each Bt and non Bt hybrids. The experimental data were pooled and subjected to statistical analysis (Gomez and Gomez, 1984).

## Results and Discussion

The experimental results revealed that the per cent egg hatching of *R. reniformis* was reduced after 24 h of exposure period in all the three Bt hybrids compared to non Bt. Jagannath fusion Bt registered the highest per cent decrease in egg hatching at 48h (29.55%) with an average of 19.55% decrease over non Bt followed by Rasi Bt and Bunny Bt (Table 1) throughout the period of experimentation. The ovicidal activity of Bt toxins might be attributed to some alteration in the permeability of egg shell allowing toxins to enter and interact with the membrane of the embryo as suggested by Abu-Dhaim *et al*. (2005) Since the Bt strains produced chitinase and penetrated the gelatinous matrix, the eggs were exposed to the Bt toxins and resulted in the inhibition of egg hatching. The present result was supported by Qasim khan *et al*. (2010) who found that Bt isolates collected from the rhizosphere of okra, brinjal, tomato, cotton, cabbage, onion and water melon crops were effective in inhibiting hatching of *Meloidogyne sp* eggs.

**Table 1. Influence of Bt cotton root exudates on egg hatching of *Rotylenchulus reniformis***

| Bt hybrid | Number of eggs hatched after exposure to root exudates at | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 24h | 48h | 72h | 96h | 120h | 144h | 168h | Mean |
| Bunny Bt | 15.07 | 20.00 | 27.92 | 31.61 | 34.69 | 36.00 | 36.38 | 28.81 |
| | (3.77) | (4.31) | (5.02) | (5.35) | (5.57) | (5.69) | (5.72) | |
| Bunny non Bt | 17.07 | 23.38 | 29.15 | 32.92 | 34.76 | 36.07 | 36.61 | 29.99 |
| | (3.81) | (4.35) | (4.82) | (5.12) | (5.29) | (5.4) | (5.50) | |
| Decrease over non Bt (%) | 11.71 | 14.40 | 4.21 | 3.97 | 0,20 | 0.19 | 0.62 | 3.93 |
| t value | 0.78 | 0.68 | 0.91 | 0.91 | 0.99 | 0.99 | 0.98 | |
| RasiBt | 26.07 | 31.84 | 37.46 | 43.53 | 44.61 | 45.84 | 46.23 | 39.36 |
| | (4.86) | (5.35) | (5.82) | (6.28) | (6.35) | (6.43) | (6.47) | |
| Rasi non Bt | 30.38 | 38.00 | 43.30 | 48.84 | 49.53 | 50.00 | 50.38 | 44.34 |
| | (5.21) | (5.82) | (6.22 | (6.59) | (6.64) | (6.66) | (6.69) | |
| Decrease over non Bt (%) | 14.18* | 16.21* | 13.48* | 10.87* | 9.93* | 8.32* | 8.23* | 11.23 |
| t value | 0.46 | 0.40 | 0.45 | 0.57 | 0.60 | 0.66 | 0.66 | |
| Jagannath fusion Bt | 15.69 | 19.07 | 25.76 | 30.76 | 32.15 | 33.46 | 33.84 | 27.24 |
| | (3.86) | (4.23) | (4.86) | (5.30) | (5.41) | (5.53) | (5.57) | |
| Jagannath non Bt | 20.84 | 27.07 | 32.38 | 37.70 | 39.30 | 39.76 | 40.00 | 33.86 |
| | (4.31) | (4.93) | (5.40) | (5.83) | (5.96) | (6.00) | (6.02) | |
| Decrease over non Bt (%) | 24.71* | 29.55* | 20.44* | 18.40* | 18.19* | 15.84* | 15.4* | 19.55 |
| t value | 0.14 | 0.07 | 0.18 | 0.23 | 0.22 | 0.29 | 0.30 | |

* Values are significant at 5 per cent level.Figures in parentheses are $\sqrt{n+0.5}$ transformed value.

Al-Banna and Khyami Horanialso (2004) reported that the *B. thuringiensis* strain at higher dose completely inhibited or significantly reduced egg hatching. But, Devidas and Rehberger (1992) stated that the Bt toxin (exotoxin) had relatively little effect on hatching of root-knot nematode eggs and only at high concentrations of the liquid preparation, there was significant reduction in hatching.In the present study the difference in per cent egg hatching between Bt and non Bt of Bunny was not significant. The insecticidal property or toxins not released

**Table 2. Influence of Bt cotton root exudates on juveniles of *Rotylenchulus reniformis***

| Bt hybrid | Per cent juvenile mortality after exposure to root exudates at | | | | | | | Mean (%) |
|---|---|---|---|---|---|---|---|---|
| | 24h | 48h | 72h | 96h | 120h | 144h | 168h | |
| Bunny Bt | 18.76 | 29.07 | 35.92 | 43.38 | 50.46 | 56.15 | 58.38 | 41.97 |
| | (23.55) | (29.51) | (31.85) | (39.46) | (44.78) | (51.14) | (56.73) | (39.57) |
| Bunny non Bt | 18.07 | 29.00 | 35.38 | 42.92 | 50.00 | 55.84 | 58.00 | 41.31 |
| | (33.10) | (23.25) | (30.20) | (34.30) | (39.18) | (50.08) | (53.49) | (37.65) |
| table value | 20.68 | 0.96 | 0.73 | 0.77 | 0.60 | 0.72 | 0.57 | |
| Rasi Bt | 18.00 | 29.00 | 35.61 | 42.23 | 48.76 | 54.69 | 58.53 | 40.97 |
| | (23.06) | (30.24) | (34.43) | (38.74) | (43.39) | (49.12) | (56.87) | (39.40) |
| Rasi non Bt | 17.76 | 28.76 | 35.00 | 41.53 | 48.07 | 53.92 | 57.92 | 40.42 |
| | (23.19) | (30.06) | (33.40) | (37.23) | (42.92) | (47.98) | (55.02) | (38.54) |
| table value | 0.89 | 0.89 | 0.67 | 0.59 | 0.58 | 0.58 | 0.46 | |
| Jagannath fusion Bt | 15.92 | 28.53 | 37.76 | 45.38 | 52.07 | 58.30 | 59.92 | 42.56 |
| | (22.04) | (30.44) | (36.21) | (41.22) | (46.27) | (54.26) | (57.37) | (41.11) |
| Jagannath non Bt | 15.84 | 28.15 | 37.00 | 45.23 | 51.69 | 57.69 | 59.07 | 42.09 |
| | (19.53) | (29.71) | (35.27) | (40.72) | (45.78) | (52.99) | (55.81) | (40.97) |
| table value | 0.95 | 0.87 | 0.69 | 0.92 | 0.82 | 0.62 | 0.37 | |

Figures in parentheses are Sin $^{-1}$ transformed values.

through root exudates of Bunny Bt carrying *Cry* 1Ac gene, might be explained as possible reason for the same as stated by Saxena *et al.* (2004).

The per cent mortality of *R. reniformis* ($J_4$) increased with time of exposure to root exudate irrespective of all the three Bt cotton. The highest per cent mortality was recorded in Jagannath fusion Bt at 168h (59.92%) followed by Rasi Bt (58.53%) and Bunny Bt (58.38%) over their non Bt.However the difference was not significant among Bt hybrids as well as Bt and non Bt cotton (Table 2). The result is in accordance with Senthilkumar *et al.* (2008) who reported that the Bt toxin had no significant influence over juveniles of *R. reniformis.* The reason for failure of Bt against *R. reniformis* might be due to bacterial endotoxins (Osman *et al.,* 1988, Crickmore, 2005 and Abd El-Moneim and Massoud, 2009) which is not a contact poison and must be ingested by the

**Table 3. Influence of Bt cotton on attraction of juveniles of *Rotylenchulus reniformis***

| Time interval | % attraction | No. of juveniles attracted to Bt and non Bt cotton | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Bunny Bt | Bunny non Bt | t value | Rasi Bt | Rasi non Bt | t value | Jagannath Bt | Jagannath non Bt | t value |
| 24h | 20 | 65.69 | 78.60 | 0.96 | 81.84 | 77.63 | 0.90 | 60.92 | 67.22 | 0.89 |
| | 40 | 25.07 | 13.38 | | 13.69 | 17.07 | | 21.53 | 23.07 | |
| | 60 | 13.53 | 09.52 | | 9.62 | 9.53 | | 11.61 | 4.15 | |
| | 80 | 04.46 | 08.30 | | 5.53 | 8.92 | | 7.23 | 8.92 | |
| | 100 | 04.60 | 04.46 | | 5.38 | 4.3 | | 3.06 | 2.6 | |
| 48h | 20 | 65.07 | 63.38 | 0.88 | 77.69 | 69.38 | 0.94 | 55.61 | 61.07 | 0.87 |
| | 40 | 21.69 | 18.60 | | 15.63 | 19.07 | | 19.21 | 14.92 | |
| | 60 | 12.46 | 10.30 | | 9.84 | 10.92 | | 13.07 | 2.23 | |
| | 80 | 04.60 | 07.52 | | 5.38 | 10.3 | | 7.52 | 10.15 | |
| | 100 | 9.53 | 12.46 | | 7.53 | 7.53 | | 8.92 | 10.6 | |
| 72h | 20 | 61.53 | 56.30 | 0.91 | 71.62 | 64.92 | 0.92 | 49.53 | 60.15 | 0.91 |
| | 40 | 20.46 | 22.60 | | 17.69 | 16.3 | | 19.07 | 12.61 | |
| | 60 | 16.61 | 14.46 | | 9.53 | 13.53 | | 11.61 | 7.23 | |
| | 80 | 05.69 | 7.52 | | 5.84 | 9.07 | | 13.21 | 12.92 | |
| | 100 | 09.06 | 13.38 | | 11.38 | 13.62 | | 10.92 | 13.07 | |
| 120h | 20 | 56.61 | 52.46 | 0.94 | 67.84 | 64.3 | 0.91 | 46.92 | 52.61 | 0.85 |
| | 40 | 21.07 | 20.60 | | 17.53 | 15.06 | | 21.07 | 20.15 | |
| | 60 | 16.46 | 10.30 | | 9.61 | 10.92 | | 13.53 | 12.92 | |
| | 80 | 09.69 | 13.38 | | 13.69 | 15.53 | | 11.21 | 7.23 | |
| | 100 | 09.53 | 17.52 | | 7.38 | 11.63 | | 11.61 | 13.07 | |
| 144h | 20 | 56.61 | 50.46 | 0.92 | 67.68 | 62.92 | 0.89 | 47.21 | 52.15 | 0.87 |
| | 40 | 17.53 | 18.60 | | 15.38 | 13.52 | | 16.92 | 18.61 | |
| | 60 | 15.07 | 14.30 | | 10.53 | 12.3 | | 13.07 | 12.92 | |
| | 80 | 11.69 | 13.38 | | 14.84 | 15.61 | | 13.53 | 7.06 | |
| | 100 | 12.46 | 17.52 | | 7.61 | 13.07 | | 13.61 | 15.23 | |
| Mean | - | 22.6 | 22.8 | - | 23.21 | 23.48 | - | 20.89 | 21.19 | - |
| %Increase over Bt | - | - | 0.87 | - | - | 1.14 | - | - | 1.41 | - |

target organism to inhibit RNA synthesis and other phenomenon (Sebesta and Horska,1970).

Different concentration of root exudates of above mentioned Bt and non Bt were tested for their influence on the attraction of juveniles of *R. reniformis* towards root bits for five days at 24 h interval. The overall per cent increase in the attraction of juveniles was high in Jagannath non Bt (1.41%) followed by Rasi (1.14%) and Bunny (0.87%) over their Bt lines. However the differences in per cent attraction of $J_4$ of *R. reniformis* between Bt and non Bt as well as among the three Bt hybrids was not significant (Table 3). The present finding was contradictory to the findings of Sikora (1988) and Sikora and Hoffmann-Hergarten (1993), who opined that Bt mechanisms include production of metabolites which reduce hatching and attraction and thereby control nematodes.

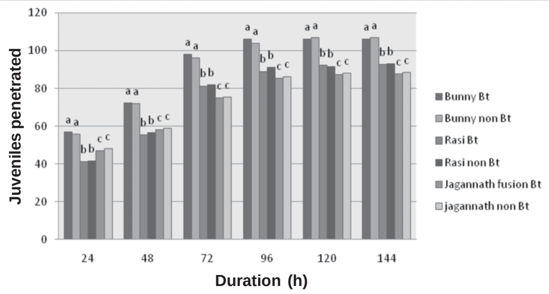


**Fig. 1. Influence of Bt cottton on penetration of *Rotylenchulus reniformis* (Bars labeled with the same letter do not differ significantly from each other (P = 0.05)).**

The study on influence of Bt cotton on root penetration revealed that the number of juveniles ($J_4$) penetrated was lesser in Bt over their respective non Bt but the difference was not significant between Bt and non Bt (Fig 1). However it was stated that the use of bacterium *B. thuringiensis* as seed treatment reduced nematode penetration in plant root system of potato and sugar beet (Racke and Sikora, 1992; Oostendrop and Sikora, 1989). Senthilkumar *et al*. (2008) also reported delay in reniform nematode penetration in Bt cotton. Similarly the nematode penetration was delayed in the present study as the

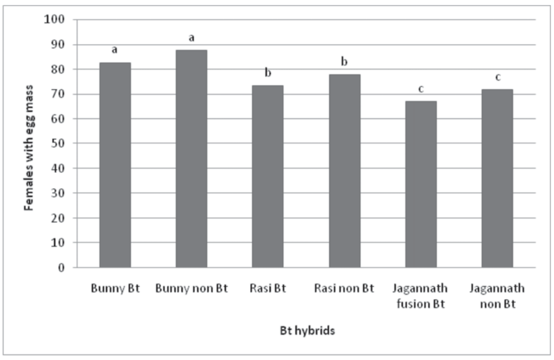


**Fig 2. Influence of Bt cotton on female population of *Rotylenchulus reniformis.* (Bars labeled with the same letter differ significantly from each other (P = 0.05)).**

number of nematodes penetrated in roots was increased with increase in days after inoculation in all the hybrids and it was maximum at 144 h after inoculation(Fig. 2).

The present study on reniform nematode penetration and development revealed that there was reduction in number of females with egg mass compared to non Bt of the same hybrid. However there was no significant difference in respect of eggs per egg mass (Fig 2 and 3). Similar result of significant reduction in egg mass of root knot nematode on Bt tomato (*Cry* 5B gene) and no significant difference in respect of eggs per egg mass was reported by Li *et al*. (2008). It was also supported by EL-Sherif *et al.* (2007) who observed reduction in number of females of *M. incognita* in eggplant treated with *B. thuringiensis* as soil application(Fig. 3).

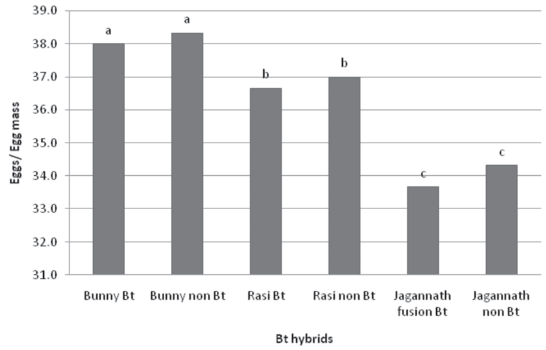


**Fig 3. Influence of Bt cotton on female fecundity of *Rotylenchulus renifromis.* (Bars labeled with the same letter do not differ significantly from each other (P = 0.05)).**

The results of the present study on the influence of Bt cotton on *R. reniformis* are inconclusive. Similar studies on other Bt cotton varieties having different genes have to be intensified for concrete confirmation about theimpact of Bt cotton against reniform nematode.

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