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Glyphosate tolerant and insect resistant transgenic Bt maize efficacy against shoot borer, cob borer and non-target insect pests

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ABSTRACT

Introducing Bt insect resistance genes into hybrid maize seems to be the most feasible and effective technique accessible to control the pest. Transgenic stack hybrid maize (MON 89034X NK 603) was developed by Monsanto for checking yield losses of maize crop to increase productivity. Transgenic stack hybrid maize was claimed to have both insect protection and herbicide tolerant traits to provide protection to the crop from target pests and also provide effective weed management. MON 89034X NK 603 is 2nd generation glyphosate tolerant with Bt maize technology effective against lepidopteron insect pests with "dual mode of action". A field experiment was carried out at ICAR-Directorate of Weed Research, Jabalpur for two years during *Kharif* (rainy season) 2009 and 2010 to evaluate the efficacy of transgenic stack hybrid maize (MON 89034X NK 603) against shoot borer (*Chilo partellus*), cob borer (*Helicoverpa armigera*), non-target insect pests and beneficial insects. Treatments consisted of two transgenic stacked hybrids named '*Hishell*' and '*'900M Gold'*' with two conventional hybrids namely '*Proagro-4640*' and '*'HQPM-1'*'. Artificial infestation of *Chilo partelus* revealed complete absence of stem borer infestation in all the transgenic entries of '*Hishell'* and *'900M Gold'* with less than one leaf injury score (LIS), while in other conventional entries, stem borer infestation was observed and LIS was more than one. *Hlicovepa armigera* infestation was also not observed on transgenic hybrids whereas in the remaining non-transgenic maize treatment, significant attack of *Helicoverpa armigera* was observed in the range of 37 to 56%. These transgenic entries were not found resistant to aphids and grass hoppers. Beneficial insects were observed to visit transgenic Bt maize and conventional maize entries with no significant difference.

Key words: *Chillo partellus*, Glyphosate tolerant maize, *Heliothis armigera*, MON 89034X NK 603. Non-target insect pest, Insect resistant maize, Transgenic maize

Food security is an important political and social agenda (Anon 2010). Global population has increased four fold during the past century with current estimates of around 9.2 billion by 2050 (UN 2009). With this projected population of about 10 billion by 2052, an immediate priority for agriculture is to achieve increased crop yields in a sustainable and cost-effective way. Insecticides introduction in 1947 paved the way to increase food production. Despite an annual pesticide budget of US $30 billion, losses owing to insects, weeds and disease for eight of the world's major crops were estimated to be in the range of US $244 billion per annum, representing 43% of world production (Oerke 2006). Paoletti and Pimentel (2000) estimated that in the absence of these synthetic pesticides, losses might increase by further 30%. However, despite pesticide contribution, it has long been recognized that such chemicals pose both environmental and health concerns. Rachel Carson (1962) cautioned the world about the overuse of pesticides in her book '*Silent Spring'*. Recombinant DNA technology to produce transgenic crops (genetically modified (GM) or engineered, biotech) with enhanced tolerance to abiotic or biotic stresses can make a significant contribution to achieve food security in the world.

Herbicide tolerant and insect resistant genetically modified (GM) crop have become leading features in agro ecosystem of many of the world's agricultural regions (ISAAA 2016). When insects and herbicide tolerant crops are employed as an integrated component of insect and weed management, productivity of crop is increased. In recent years, it has become evident that insect-resistant crops expressing δ-endotoxin genes from *Bacillus thuringiensis* have made a significant beneficial influence on global agriculture in terms of better

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quality of produce and pests decrease (Gatehouse *et al*. 2011, ISAAA 2016). *Bacillus thuringiensis*, commonly known as Bt, is a bacterium that occurs naturally in the soil. However, because of the potential for pest populations to evolve resistance, and owing to lack of effective control of pests, alternative strategies are being developed. Some of these are based on *Bacillus* spp. or other insect pathogens, while others are based on the use of plant and animal-derived genes. But, if such techniques are to play a beneficial role in crop security, it is desirable that they do not have a negative impact on beneficial organisms. This widely held fear over the environmental and biological impacts of GM crops has led to the extensive examination of transgene proteins on non-target and beneficial insects (John *et al*. 2001, Gatehouse 2011). The introduction of insect-resistant Bt Cotton for commercial production in 2002 turned out to be a large success, which is reflected that biotech cotton was sown globally in 22.3 million hectares of land in 2016 (ISAAA 2016). Bt cotton has reduced the dependency on chemical pest control, increased yields and profits for smallholder farmers in a sustainable way over a long period, and has thereby contributed to a positive economic and social development in India (Kathage and Qaim 2012, Qaim and Kouser 2013).

Maize (*Zea mays* L.) is considered a promising option for diversifying agriculture in upland areas of India. It now ranks as the third most important food grain crop. In India, maize is grown in a wide range of situations, extending from extreme semi-arid to sub-humid and humid regions. Bihar, Madhya Pradesh, Rajasthan and Uttar Pradesh are traditional maize growing areas while Karnataka and Andhra Pradesh are non-traditional maize areas. Maize crop is faced with some biotic and abiotic limitations to attain the target production. Proper management of pests can only make the production to the required levels. Insects and weeds are the major restraints to lower down the production. Among these, insects may cause huge yield losses in the range of 25-50%, if not managed properly. The development of insect and weed tolerant maize is a step to boost maize production. The 'insect resistant maize' in combination with "Rounup Ready (glyphosate tolerant)' have been genetically engineered to resist the attack of some problematic insects like shoot and cob borer and allow spraying of herbicide glyphosate on both crop and weeds without harming the crop but to kill all types of weeds. This dual action strategy will help to reduce cost and increase yield. Out of 60.6 million hectares of biotech maize sown in the world, comprised of 6 million hectares insect resistant (IR), 7 million hectares herbicide tolerant (HT) and 47.7 million hectares (IR/HT) insect resistant/herbicide tolerant (ISAAA 2016).

Post-emergence application of glyphosate at 900, 1800 and 3600 g/ha registered lower weed density, dry weight and higher weed control efficiency in transgenic '*Hishell*' and '*900 M Gold*' maize hybrids in the Maize Trial I and post-emergence application of glyphosate at 900 and 1800 g/ha registered lower weed density, dry weight and higher weed control efficiency in transgenic '*30V92*' and '*30B11*' hybrids in the Maize Trial II compared to their state and national checks (Chinnusamy *et al*, 2014). In our earlier study in evaluation of bioefficacy of glyphosate tolerant transgenic maize (MON 89034X NK 603) under field conditions, we found lower weed density and higher weed control efficiency (100%) in all transgenic maize hybrids at 21 DAS and at harvest and three times high yield than the normal hybrid (Dixit *et al.,* 2016). Simultaneously, another combined experiment was done to study the bioefficacy of Bt in transgenic maize (MON 89034X NK 603) against lepidopteron pests, secondary pests, non-target insect pests and on beneficial insects and its effect on conventional counterpart hybrids. This paper presents the results of efficacy of glyphosate tolerant transgenic stack maize with it, lepidopteran insect resistant Bt maize on major insect pests like *Chillo partellus, Helicoverma armigera*, non-target insect pests and beneficial insects.

MATERIALS AND METHODS

A field experiment was conducted for consecutive two years at research farm ICAR-Directorate of Weed Research, Jabalpur, Madhya Pradesh (India) during *Kharif* (rainy season) 2009 and 2010 under Bio-safety Research Trial Level-1 for transgenic staked maize hybrids to evaluate the bio-efficacy of Bt in transgenic maize hybrids (MON 89034 X NK 603) against weeds, key maize insect pests, secondary pests, non-targeted insect pests and beneficial insects. The climate of the area was typically sub-humid and sub-tropical with an average annual rainfall of 1253 mm. The geographical location of the experiment was situated at 23°10'N latitude and 79° 57'E longitude with an altitude of 412 m above MSL in Kymore plateau and Satpura hills of Madhya Pradesh, India. The soil of the Directorate's farm is medium black (Typic Haplustert) and moderately alkaline with the organic carbon (0.9%), available N (177 kg/ha), P (8 kg/ha), K (478 kg/ha), sulphur (35 ppm), Zn (0.7 ppm), EC of saturated extract (0.18 dS/m at 25 °C) and neutral pH of 6.3. Experiment was done with 16 treatments in randomized block design (RBD) replicated thrice. The gross plot size was 18 m^2 (5 × 3.6 m). The spacing between the rows and plants were 60 and 25 cm, respectively. The recommended dose of 150:75:75 kg of NPK/ha were given in the form of urea, diammonium phosphate and muriate of potash. As per protocol, aerial isolation distance of 300 m was maintained in periphery of experimental area.

The transgenic staked maize hybrids '*Hishell'* (MON 89034xNK 603) and '*900 M Gold*' (MON 89034xNK 603), and conventional hybrids namely '*Proagro-4640*' and '*'HQPM-1'*' were sown on July 7 in 2009 and 2010 during *Kharif* season with university recommendation for weed and insect protection (P), no weeding from sowing to harvest with no chemical insect protection (control), and no weeding from sowing to harvest with only chemical insect protection (control), national check conventional (P), national check conventional control, local check conventional (P), and local check conventional (control).

In want of natural infestation of major pest of maize *Chilo partelus* in the area, artificial inoculation was done to assess the resistance against stem borer incidence. Eggs of *Chilo partellus* were obtained from International Crops Research Institute for the Semi-Arid Tropics (*ICRISAT*), Hyderabad (India). Egg card with 10-12 eggs were tagged in each whorl of 5 plants from border rows at randomly in each treatment after 40 days of sowing. The observations on per cent infestation of stem borer and mean leaf injury score (LIS) were taken after 15 days of inoculation. All the plants showing dead-heart formation from four inner lines were counted and their percentages was calculated on the basis of total plant stand. Such dead hearts were ascertained whether they are actually caused by stem borer or by other insects. All the plants showing symptoms of borer damage or shot holes were counted and their percentages were calculated on the basis of total plant stand. Injury scale was taken at 1 to 10 scale as follows: 1. Apparently healthy plant, 2. Plant showing slight damage pinholes on 1-2 leaves, 3. Plant showing slight damage pinholes on 3-4 leaves, 4. Plant showing injury pinholes, shot holes slit in about 1/3 total leaves, 5. Plant showing 50 % leaf damage, 6. Plant showing 2/3 total leaf injuries, 7. Plant with every type of injury almost all damaged, 8. Entire plant with complete leaf injury likely to form dead heart, 9. Complete dead heart. Mean leaf injury score/ plot was calculated based on total leaf injury score (LIS) divided by total no. of plants scored. The plants on which leaf injury was scored, were selected for calculating stem tunneling. Per cent stem tunneling was calculated at the time of harvest by calculating total borer tunneled length divided by plant height of affected plants. Average per cent stem tunneling per plot was calculated by dividing total length by no. of plants taken for tunneling observations.

There was no secondary major pest on the maize crop during experimental period, therefore to see the infestation level on transgenic and conventional entries, artificial inoculation of about 8-10 first instar larvae of *Helicoverpa armigera* (an destructive insect pests of cobs of maize) was made on each 5 plants of transgenic and conventional entries at silken stage of cobs. Per cent infestation of *Helicoverpa armigera* was recorded at 90 days after sowing. All the non-target insect species visiting the various treatments other than secondary lepidopterans and beneficial insects were recorded at 30, 45 and 60 days after sowing (DAS) from 10 plants/treatment. Population of beneficial predators, pollinators were recorded per plant at 30, 45 and 60 DAS on 10 plants/treatment. In University recommendation plots, for insect control, application of endosulfan 35 EC was done each year at 17 DAS and weed control with use of atrazine at 1.0 kg/ha as pre-emergence.

The data on various observations recorded during course of investigation were analyzed statistically by adopting the procedure described by Gomez and Gomez (1984). The data were subjected to Fisher's method of analysis of variance and the level of significance used in F test was P = 0.05. The critical differences were calculated at 5% probability level whenever F values was found to be significant. Wherever it was necessary, the original values were transformed using arc-sin transformation.

RESULTS AND DISCUSSION

Natural infestation

No natural infestation of insect pests was observed in transgenic treatments, but it occurred in non-transgenic treatments. There was a mild infestation up to 20 days in conventional '*900 Gold*', national check and local check (*'HQPM-1'*), which increased significantly in some of the non-transgenic treatments when crop stage advanced from 20 to 40 DAS (**Table 1**).

Artificial infestation of *Chillo partellus*

Artificial inoculation of stem borer *Chillo partellus* was nil up to 55 DAS and leaf injury score (LIS) was less than one in all the transgenic entries of '*Hishell'* and *'900M Gold'*. In all other conventional entries, stem borer infestation was observed and the LIS was more than one. There was about 31 to 43% infestation in conventional '*900 Gold*' while in local check conventional, it was 100% (*'HQPM-1'*) followed by national check (*Proagro 4640*). Tunnel length taken at the harvest time after tearing the stems also revealed highest tunnel length in national check and local check, which correlated highest infestation per cent of stem borer after artificial inoculation (**Table 2**). The Central Compliance Committee (CCC) also visited the site of trial on 18.10.2010 and monitored the insect attack and was convinced with the results.

Artificial infestation of *Helicoverpa armigera*

No infestation of *Helicoverpa armigera* in all transgenic entries of '*Hishell'* and *'900M Gold'* was observed. Whereas, in the remaining non-transgenic maize treatments, significantly higher infestation was observed as compared to transgenic hybrids. Significantly 36 to 71% infestation was observed in all the non-transgenic lines, which showed that transgenic entries are resistance to *Helicoverva* armigera (**Table 3**).

Other insect pests

A few insect species like leaf hoppers and aphids were observed at 30 and 60 days after sowing. Aphid population was observed on both transgenic and non-transgenic lines, which indicated that the transgenic lines are equally susceptible to aphids. The trend of population decline at 60 days was also same in both transgenic and non-transgenic line. Leaf hoppers were found to attack only a few non-transgenic lines at 60 days (**Table 4**).

Beneficial insects

Data on beneficial insects like coccinalids, spiders were taken at 30, 45 and 60 DAS. Good number of adults of predator *Coccinella septempunctata* was observed at 30 DAS in both transgenic and non-transgenic maize hybrid, which declined sharply at 60 DAS in both the lines (**Table 5**). The Syrphids were also observed on both the lines at 30 DAS. Like-wise, pollinators like honey bees were also recorded at 30, 45 and 60 DAS. The spiders were also observed at 30, 45 and 60 DAS on transgenic and non-transgenic treatments. These observations showed that beneficial insect species can thrive well on transgenic lines also in addition to conventional lines (**Table 5**).

Several species of insects that attack maize, also feed on maize pollen. Therefore, study done by

Table 1. Natural infestation of stem borer (*Chilo partellus*) and mean leaf injury score (LIS 1-9 scale) at 20 and 40 DAS in transgenic and conventional maize hybrids

Treatment	Natural infestation at 20 DAS	Mean leaf injury score at 20 DAS	Natural infestation at 40 DAS	Mean leaf injury score at 40 DAS
Hishell (MON 89034xNK 603) + round up 900 g/ha	0.71 (0.00)	1.00	0.71 (0.00)	1.00
Hishell (MON 89034xNK 603) + round up 800 g/ha	0.71 (0.00)	1.00	0.71 (0.00)	1.00
Hishell (MON 89034xNK 603) + round up 3600 g/ha	0.71 (0.00)	1.00	0.71 (0.00)	1.00
900 M Gold (MON 89034xNK 603) + round up 900 g/ha	0.71 (0.00)	1.00	0.71 (0.00)	1.00
900 M Gold (MON 89034xNK 603) + round up 1800 g/ha	0.71 (0.00)	1.00	0.71 (0.00)	1.00
900 M Gold (MON 89034xNK 603) + round up 3600 g/ha	0.71 (0.00)	1.00	0.71 (0.00)	1.00
Hishell conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 250 g/ha	0.91 (0.41)	1.00	0.91 (0.41)	1.07
Hishell conventional (control)	0.91 (0.41)	1.00	0.91 (0.41)	1.07
Hishell conventional (control) + endosulfan 35 EC 1250 g/ha	0.71 (0.00)	1.00	0.71	1.13
900 M Gold conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 1250 g/ha	1.39 (1.67)	1.00	0.91 (0.41)	1.07
900 M Gold conventional (control)	0.71 (0.00)	1.00	0.71 (0.00)	1.20
900 M conventional (control) + endosulfan 35 EC 1250 g/ha	1.05 (0.83)	1.00	0.91 (0.41)	1.07
National check conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 1250 g/ha	1.39 (1.67)	1.07	0.71 (0.00)	1.27
National check conventional control	1.05 (0.83)	1.00	0.71 (0.00)	1.47
Local check conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 1250 g/ha	1.94 (3.33)	1.60	1.96 (3.35)	2.53
Local check conventional (control)	1.36 (1.67)	1.40	1.16 (1.26)	2.07
LSD (p=0.05)	0.57	0.17	0.44	0.48

Original values are given in parentheses subjected to arc sine

Table 2. Effect of artificial infestation of stem borer (*Chilo partelus*) and mean leaf injury score (LIS 1-9 scale) at 15 DAI (days after inoculation) in transgenic and conventional maize hybrids

Treatment	Infestation at 55 DAS (%)	Mean leaf injury (LIS) score at 55 DAS	Tunnel length (cm) at harvest
Hishell (MON 89034xNK 603) + round up 900 g/ha	4.05 (0.00)	1.00	0.00
Hishell (MON 89034xNK 603) + round up 800 g/ha	4.05 (0.00)	1.00	0.00
Hishell (MON 89034xNK 603) + round up 3600 g/ha	4.05 (0.00)	1.00	0.00
900 M Gold (MON 89034xNK 603) + round up 900 g/ha	4.05 (0.00)	1.00	0.00
900 M Gold (MON 89034xNK 603) + round up 1800 g/ha	4.05 (0.00)	1.00	0.00
900 M Gold (MON 89034xNK 603) + round up 3600 g/ha	4.05 (0.00)	1.00	0.00
Hishell conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 250 g/ha	39.23 (40.00)	3.20	1.63
Hishell conventional (control)	26.56 (20.00)	2.13	2.47
Hishell conventional (control) + endosulfan 35 EC 1250 g/ha	30.79 (26.66)	2.60	1.63
900 M Gold conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 1250 g/ha	23.28 (20.00)	2.67	1.97
900 M Gold conventional (control)	30.79 (26.66)	2.73	1.80
900 M conventional (control) + endosulfan 35 EC 1250 g/ha	43.08 (46.66)	3.33	2.23
National check conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 1250 g/ha	35.00 (33.33)	3.27	1.77
National check conventional control	39.23 (40.00)	3.27	3.13
Local check conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 1250 g/ha	85.94 (100.00)	7.00	3.40
Local check conventional (control)	78.44 (93.33)	5.93	3.63
LSD (p=0.05)	10.58	0.94	0.99

Table3. Effect of artificial infestation of cob borer (*Helicoverpa armigera*) at 90 DAS in transgenic and conventional maize hybrids

Treatment	Natural infestation	Artificial infestation at 90 DAS (%)
Hishell (MON 89034xNK 603) + round up 900 g/ha	0.00	0.71 (0.00)
Hishell (MON 89034xNK 603) + round up 800 g/ha	0.00	0.71 (0.00)
Hishell (MON 89034xNK 603) + round up 3600 g/ha	0.00	0.71 (0.00)
900 M Gold (MON 89034xNK 603) + round up 900 g/ha	0.00	0.71 (0.00)
900 M Gold (MON 89034xNK 603) + round up 1800 g/ha	0.00	0.71 (0.00)
900 M Gold (MON 89034xNK 603) + round up 3600 g/ha	0.00	0.71 (0.00)
Hishell conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 250 g/ha	0.00	7.98 (63.33)
Hishell conventional (control)	0.00	7.31 (53.33)
Hishell conventional (control) + endosulfan 35 EC 1250 g/ha	0.00	8.06 (65.00)
900 M Gold conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 1250 g/ha	0.00	7.34 (55.00)
900 M Gold conventional (control)	0.00	6.07 (36.66)
900 M conventional (control) + endosulfan 35 EC 1250 g/ha	0.00	7.53 (56.66)
National check conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 1250 g/ha	0.00	7.75 (60.00)
National check conventional control	0.00	7.76 (60.00)
Local check conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 1250 g/ha	0.00	8.39 (70.00)
Local check conventional (control)	0.00	8.47 (71.66)
LSD (p=0.05)	0.00	1.08

Original values are given in parentheses

Table 4. Population of other pest species on transgenic and non-transgenic lines

Treatment	Aphids 30 DAS	Leaf hoppers 60 DAS
Hishell (MON 89034xNK 603) + round up 900 g/ha	1259.00	0.00
Hishell (MON 89034xNK 603) + round up 800 g/ha	586.00	0.00
Hishell (MON 89034xNK 603) + round up 3600 g/ha	978.33	0.00
900 M Gold (MON 89034xNK 603) + round up 900 g/ha	1199.33	0.00
900 M Gold (MON 89034xNK 603) + round up 1800 g/ha	1145.00	0.00
900 M Gold (MON 89034xNK 603) + round up 3600 g/ha	1073.33	0.00
Hishell conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 250 g/ha	1211.33	0.00
Hishell conventional (control)	1073.00	0.00
Hishell conventional (control) + endosulfan 35 EC 1250 g/ha	856.33	2.00
900 M Gold conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 1250 g/ha	1421.66	1.33
900 M Gold conventional (control)	1342.67	0.67
900 M conventional (control) + endosulfan 35 EC 1250 g/ha	1140.00	1.00
National check conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 1250 g/ha	1417.67	0.00
National check conventional control	1698.33	0.00
Local check conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 1250 g/ha	1433.33	0.33
Local check conventional (control)	1533.67	0.00
LSD (p=0.05)	NS	0.95

Pilcher *et al.* (1997) showed that direct consumption of transgenic maize pollen by immature stages of three predatory species commonly found in maize fields did not affect development or survival. The mortality rate of nymphal stages of *Orius majusculus* (predator) was the same when fed a thrips species reared on Bt maize as and when the thrip were fed on non-Bt maize (Zwahlen *et al.* 2000). However, increased mortality of lacewing (*Chrysoperla carnea*) larvae was observed when the larvae fed on an artificial diet containing Bt toxin or preyed on maize borers or other lepidopteran larvae that had fed on transgenic maize (Hilbeck *et al.*1998). Resende *et al.* (2016) assessed the effect of the cultivation of genetically modified crops in Brazil on non-target insect diversity by comparing a homogeneous maize field with conventional and transgenic maize, conveying different Bt proteins in seven counties of Minas Gerais, Brazil. The results did not support the hypothesis that Bt protein affects insect biodiversity. Romeis *et al.* (2014) found that there is sufficient information available today to conclude that Bt maize containing Cry 1Ab does not harm beneficial insect predator *Chrysoperla carnea* (Neuroptera: Chrysopidae). Our observation also revealed the presence of beneficial predator on transgenic and non-transgenic entries. Therefore, one advantage of the use of GM maize would be in reduction of insecticide applications, especially of broad spectrum type (Dively 2005, Naranjo 2005). In many cases, the insect richness estimated for conventional and Bt maize fields was not significantly different or was lower than on Bt maize. Again, considering that conventional maize fields underwent insecticide spraying, it is likely that the low insect population in these fields is the result of this impact. However, although other studies have already shown that the effect of insecticide use may be stronger on insect communities than the impacts of transgenic *Bt* crops. In the present study, the estimated insect population was not significantly affected by insecticide use on the studied maize fields (Dively 2005). The use of transgenic plants may be considered as one more method for integrated pest management (IPM). Regarding community diversity, the presence of secondary pests was directly related with the richness of natural enemies. Recent literature also indicate no significant effect of Bt proteins on natural enemies.

Table 5. Population of beneficial insects at 30, 45 and 60 days after showing (DAS)

Treatment	Coccinalid DAS			Spider DAS			Syrphid			Pollinator		
	30	45	60	30	45	60	30	45	60	30	45	60
Hishell (MON 89034xNK 603) + round up 900 g/ha	8.33	3.67	0.00	2.00	1.67	3.00	9.00	9.67	3.33	3.00	2.33	3.00
Hishell (MON 89034xNK 603) + round up 800 g/ha	9.33	4.00	0.00	0.00	0.67	2.00	8.00	8.67	2.33	2.00	2.00	1.67
Hishell (MON 89034xNK 603) + round up 3600 g/ha	7.33	2.67	0.00	0.30	2.67	2.33	9.00	17.00	2.00	1.00	2.00	1.67
900 M Gold (MON 89034xNK 603) + round up 900 g/ha	9.33	5.33	0.00	1.05	1.00	2.67	11.0	11.67	2.33	1.33	1.33	2.33
900 M Gold (MON 89034xNK 603) + round up 1800 g/ha	6.00	5.00	0.00	0.67	1.33	2.67	8.33	17.7	1.67	1.33	3.33	1.67
900 M Gold (MON 89034xNK 603) + round up 3600 g/ha	5.00	4.33	0.00	0.33	.33	2.00	16.00	16.7	1.67	2.00	3.00	2.67
Hishell conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 250 g/ha	12.3	6.33	0.00	1.00	2.33	2.33	13.00	11.7	2.00	0.67	2.67	3.33
Hishell conventional (control)	6.33	3.67	0.67	1.09	2.00	1.33	14.33	11.0	2.67	0.67	2.00	2.33
Hishell conventional (control) + endosulfan 35 EC 1250 g/ha	10.33	3.67	0.00	1.67	1.00	2.67	12.00	12.3	2.00	3.33	1.67	2.67
900 M Gold conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 1250 g/ha	9.33	4.67	0.67	1.22	2.00	2.33	19.33	28.7	2.00	3.67	3.00	2.67
900 M Gold conventional (control)	7.33	2.33	0.00	0.33	0.67	2.33	9.00	10.7	2.00	3.00	2.00	2.00
900 M conventional (control) + endosulfan 35 EC 1250 g/ha	11.33	4.33	0.67	0.0	0.33	2.67	10.00	9.3	3.33	3.00	3.33	2.67
National check conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 1250 g/ha	11.67	7.67	0.67	0.0	2.00	1.67	11.33	18.3	1.00	2.00	3.67	2.33
National check conventional control	12.67	6.33	0.67	0.0	1.00	1.67	13.33	14.33	1.67	6.00	6.00	3.00
Local check conventional (P) + atrazine 1000 g/ha and endosulfan 35 EC 1250 g/ha	8.33	2.67	0.00	1.67	067	1.33	12.33	7.67	1.67	5.67	0.67	2.00
Local check conventional (control)	8.67	3.67	1.00	0.33	1.00	2.00	10.67	7.67	1.00	5.67	5.33	2.67
LSD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

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