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Effects of refuge crops and sowing dates on the growth and yield performance of Bollgard II variety of cotton (*Gossypium hirsutum* L.) in Allahabad condition

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Abstract
Extensive use of pesticides and insecticides to control various kinds of pests and bollworms in *Bt* (*Bacillus thuringiensis*) cotton to create ecological imbalance in the environment. For managing that natural refuge crops helps to delay resistance in *Bt* cotton. An experiment was conducted to estimate the effect of refuge crop and sowing dates on growth and yield performance of Bollgard II variety of *Bt* cotton in two different Departmental Fields at Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad (Uttar Pradesh) during 2016. When alternatively sown 50% *Bt* with 50% refuge *nBt* cotton, significantly early planting cotton of first field gave more number of monopodial branches plant^{-1}(2.55) sympodial branches plant^{-1} (17.00) than the refuge *nBt* cotton whereas plant height (cm) was found maximum in 50% refuge *nBt* cotton (139.33) and yield attributes was found maximum in 50% *Bt* than 50% *nBt*. In case of planting date, early planting cotton during mid march gave significantly more plant height (130.44 cm), monopodial branches plant^{-1} (2.50), sympodial (fruiting) branches plant^{-1} (26.00), bolls plant^{-1} (27.55), Average weight of bolls plant^{-1} (3.38), No. of seeds plant^{-1} (511.55) and lint weight (g) plant^{-1} (19.07) than late sowing during mid May under different cultivars.

Keywords: *Bt* cotton, resistance, refuge crops, sowing date, growth and yield attributes

Introduction
Chemical insecticides and pesticides usage during last 40 years guaranteed a production increase in agriculture and it has adversely affects our environment [1]. Extensive uses of pesticides led to contamination of water and food sources, and poisoning of non-target or beneficial insects and developed resistant against the various toxic chemicals [2, 3]. So it is necessary to introduce new technology at genetic level, which increases the quality and quantity of cotton.

By adopting new technology eliminates the risk of hazards of toxic chemicals. Transgenic cotton expressing insecticidal proteins which are isolated from the soil bacterium, *Bacillus thuringiensis*, (*Bt*) cultivated on a large scale in many countries including U.S.A. [4] Australia [5] and China [6]. In India, Indian Government legally permitted the commercial cultivation of genetically modified crops during March 2002. The *Bt* gene produces proteins that are toxic to the bollworms [7]. The *Bt* cotton varieties produced profitable yields comparable to that of conventional varieties of cotton [8].

Insecticides Resistance Management strategies helps in managing against various pests by identifying appropriate methods so as to delay resistance, make sure efficient control against target pests [9]. A strategy followed in field for delaying resistance, out crossing of non-*Bt* cotton in refuge manner with transgenic cultivar of cotton [10]. *Bt* cotton hybrids and expressing Cry1Ac and Cry2Ab delta endotoxin have been reported to be highly effective against various bollworm complex of cotton [11]. A recent estimation indicated that the loss of U.S $ 1.0 billion caused by *Helicoverpa armiger*, every year [12].

Sowing dates affects significantly the growth and yield attributes in *Bt* cotton. Early sowing crops avoiding inclement weather conditions commonly associated with the summer season which creates higher humidity and higher night temperature resulting in accelerated rates of fruit loss and abortion [13]. Delayed sowing decreased the yield and fibre traits due to reduced fruiting period and delayed maturity than the normal sowing date [14]. Optimum sowing date for a cultivar in a region is considered to be the most important and manageable factor in cotton crop [15]. Early sowing gives higher growth and yield potential than the late planting crop difficult to manage resulting lower seed cotton yield [16].

Materials and Methods

Experimental location and meteorological information

During 2016, the cotton crop was sown on well prepared ridges on 15 mid March and 15 mid May at the two different departmental fields, study was carried out at Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad (20º15'42'' N, 60º 50'31''E and 98 m above sea level) Uttar Pradesh. The experimental site was dominant with sandy loam soil and high level of nitrogen (N) and potassium (K) and low in available phosphorus (P). The pH of soil is slightly acidic in nature. The climatic conditions of Allahabad comes under subtropical belt of south east of Uttar Pradesh which experiences extremely very hot summer and fairly cold winter. The total received rainfall during crop period was 702.85 mm about which 5% are received during November to April. The rainfall was very scanty in nature during crop season particularly from boll formation to boll opening which drastically reduced the final yield of cotton.

Experimental methods

The experiment was laid out in completely randomized design by making ridges for each treatment, in two departmental fields, with different cultivars (V_1:50% *Bt*, V_2: 50% *nBt* acts as refuge crops) at different sowing dates (D_1: Mid March, D_2: Mid May). Each field contain 10 rows and 10 columns, were maintained at a spacing of 0.60 m x 0.60 m with alternatively sown *Bt* and *nBt* cotton.

Materials and cultural operations

The seeds of *Bt* cotton Bollgard II (KCH14K59) of Jaddu seeds its *nBt* variety and was obtained from the local market of Andhra Pradesh. The field was ploughed twice with tractor and harrowed with a cultivar before sowing. Before sowing in the field contained one full amount of an organic source of Field yard manure (FYM) 1.25 kg/m^2, Phosphorus was applied in the form of DAP at 8.7 g/m^2, urea as source of nitrogen at 14g/m^2, and MOP as a source of potassium, at 6.6 g/m^2 area. No pesticides were used during the small scale field trail. Entire manure was applied at the time of sowing. The experimental plots were irrigated at 15-20 days interval till the crop maturity. Since there was sufficient rain in July and August during 2016, scheduled irrigations were not given in the respective months. Hand weeding was followed to remove the weeds at 20-25 days interval. Bolls were harvested in six picking and other cultural operations were adopted throughout the growing period uniformly in all treatments.

Data collections and Analysis

For recording agronomic characters three tagged plants selected randomly from each treatment when seedling has been found and observations were recorded for plant height (cm), monopodial (vegetative) branches plant^{-1}, sympodial (fruiting) branches plant^{-1}, total boll plant^{-1}, Average boll weight (g) plant^{-1}, seed plant^{-1}, and weight of lint (g) plant^{-1}. All the collected data for Bollgard II variety of 50 % *Bt* with 50% *nBt* refuge cotton at different dates of sowing were subjected to one-way ANOVA test. All the statistical analysis were performed by using Wasp software package [17].

Results and Discussion

Plant height (cm): Analysis of variance indicated that sowing dates and different cultivars affects significantly plant height (cm) during 2016. Plant height was found higher in V_2: 50% *nBt* refuge cotton (139.33, 119.77) and lower plant height was recorded in 50% *Bt* cotton (134.66, 117.22) in both fields at different sowing dates (Table 1). The probable reason for that might due to variations found in genetic constitution of the different cultivars [18, 19]. It is also evident from the result that sowing during early planting date D_1: Mid March (130.44) of first field gives significantly maximum plant height while late planting date D_2: mid May (121.33) of second field showed minimum plant height in due to variation in temperature and short growth period [20, 21].

Monopodial branches plant^{-1}: Monopodial branches plant^{-1} is indication of its potential for higher yields in *Bt c*otton. Analysis of data given in Table 1. indicated that significantly more number of monopodial branches plant^{-1} was recorded in V_1: 50% *Bt* (2.55, 1.50) and minimum was found in V_2: 50% refuge *nBt* cotton (1.66, 1.00) respectively. Higher number of vegetative branches were observed in WCCV-48 which is *nBt* cotton than MRC 7201 (*Bt*) Bollgard II variety [22]. When compared different sowing dates maximum branches plant^{-1} was noticed in D_1: mid March (2.50) of first field than planted in D_2: Mid May (1.75) of second field. More monopodial (vegetative) branches plant^{-1} was produced during early than late planting date [23].

Table 1: Plant height (cm), monopodial branches plant^{-1} and sympodial branches plant^{-1} in Bollgard II variety of cotton as influenced by different cultivar or presence of refuge crops (V_1 and V_2) at different sowing dates (D_1 and D_2) in two different field observations during 2016.

Treatment	Plant height at harest (cm)		Monopodial branches plant^{-1}		Sympodial branches plant^{-1}	
Cultivars	2016 (F_1) D_1	2016 (F_2) D_2	2016 (F_1) D_1	2016 (F_2) D_2	2016 (F_1) D_1	2016 (F_2) D_2
V_1: 50% *Bt* cotton	134.66	117.22	2.55	1.50	17.00	12.33
V_2: 50% refuge *nBt* cotton	139.33	119.77	1.66	1.00	10.38	6.33
Mean	136.99	118.49	2.10	1.25	13.69	9.33
SEm±	0.710	0.451	0.134	0.122	0.847	0.622
CD(P=0.05)	1.497	0.921	0.473	0.430	2.980	2.188
CV%	2.517	1.413	6.400	9.798	6.207	6.675
Sowing Dates (D)						
D_1: Mid March	130.44		2.50		26.00	
D_2: Mid May	121.33		1.75		15.33	
SEm±	1.704		0.122		1.544	
CD(P=0.05)	5.988		0.434		5.425	
CV%	1.354		5.810		7.472	

Table 2: Number of bolls plant^{-1}, average weight of bolls (g) plant^{-1} number of seeds^{-1} and weight of Lint (g) plant^{-1} in Bollgard II variety of cotton as influenced by different cultivar or presence of refuge crops (V_1 and V_2) at different sowing dates (D_1 and D_2) in two different field observations during 2016.

Treatment	No. of Bolls plant^{-1}		Average weight of Bolls (g) plant^{-1}		No. of seeds plant^{-1}		Weight of Lint (g) plant^{-1}	
Cultivars	**2016 (F_1) D_1**	**2016 (F_2) D_2**	**2016 (F_1) D_1**	**2016 (F_2) D_2**	**2016 (F_1) D_1**	**2016 (F_2) D_2**	**2016 (F_1) D_1**	**2016 (F_2) D_2**
V_1: 50% *Bt* cotton	30.21	18.66	3.64	3.28	560.44	476.33	23.27	18.79
V_2: 50% refuge *nBt* cotton	21.22	10.55	3.12	2.80	281.55	201.77	11.20	7.68
Mean	25.71	14.60	3.38	3.04	420.99	339.05	17.23	13.23
SEm±	1.078	0.272	0.100	0.118	0.826	1.923	0.631	0.789
CD(P=0.05)	3.791	0.954	0.348	0.418	2.904	5.757	2.218	2.775
CV%	4.195	1.859	2.928	3.909	0.196	0.567	3.662	5.996
Sowing Dates (D)								
D_1 : Mid March	27.55		3.38		511.55		19.07	
D_2 : Mid May	18.44		3.07		402.33		10.75	
SEm±	0.681		0.096		2.454		1.358	
CD(P=0.05)	2.395		0.225		8.623		4.772	
CV%	2.965		1.988		0.537		9.107	

Sympodial branches plant^{-1}: Sympodial branches plant^{-1} is an important character for achieving good yields in cotton. It was significantly affected due to different cultivars in which *nBt* acts as refuge crops and different sowing date in Bollgard II variety of *Bt* cotton. Analysis of variance presented in table 1 indicated that V_1: 50% *Bt* cotton (17.00, 12.33) gives higher number of sympodial branches plant^{-1} than V_2: 50% *nBt* refuge cotton (10.38, 6.33) of two different fields sowing during different sowing dates. Significantly less number of sympodial branches was found in *nBt* cultivar than *Bt* cultivar [24]. These variations are due to different genetic governed traits, high temperature and environmental changes reduces the number of nodes resulting reduction in number of sympodial branches plant^{-1}. Significantly maximum fruiting branches plant^{-1} was recorded in early planting date which was D_1: mid march (26.00) against late planting during D_2: mid May (15.33) respectively.

Number of bolls plant^{-1}: Number of bolls plant^{-1} is an important yield contributing parameter to estimate the yield of seed cotton. Number of bolls plant^{-1} was significantly affected by sowing dates and different cultivars (Table 2). Comparison between treatments means showed maximum number of bolls plant^{-1} was attained in V_1: 50% *Bt* (30.21, 18.66) over V_2: 50% *nBt* refuge cotton (21.22, 10.55) of two different fields, sowing at different dates. These variations are found due to *Bt* cotton retained more number of bolls by virtue of inbuilt protection of fruiting bodies against various bollworm infestations, which damage greater number of bolls in *nBt* cotton because there is no such gene is present for protection. Presence of *nBt* cotton also delay resistance in *Bt* cotton against bollworm problems [24, 25]. Shedding of squares and young bolls ranged from 75 to 80% across the cultivars. Fruiting forms shed due to entomological factors accounted for 20 % in *Bt* and 50% *nBt* cotton respectively [26].

Differ in planting dates influenced significantly the number of bolls plant^{-1}. Results showed in Table 2. indicated that more number of bolls plant^{-1} was found in early planting date D_1: Mid March (27.55) and less was observed in mid May (18.44) because the temperature was high during May, more shedding of bolls, and heavily infested with pest problems. Temperature effects on cotton reproductive development stages by growing cotton under natural condition and regulated growth chambers. Their work revealed that fruit retention declined quickly when mean temperature was high than 28º C [27, 28].

Average boll weight (g) plant^{-1}: Average boll weight (g) plant^{-1} was significantly affected by different cultivar and sowing dates treatments. Maximum average boll weight plant^{-1} value was noted in V_1: 50% *Bt* (3.64, 3.28) in comparison to V_2: 50% refuge *nBt* (3.12, 2.80) cotton in different field observation at different sowing dates (Table 2). These variations due to different cultivars had different genetic makeup or *Bt* cotton perform better due to inbuilt resistance against bollworm by the presence of *Bt* gene which in turn to move in reproductive phase early by excessive vegetative growth and produce more seed cotton yield. Refuge *nBt* cotton helps to delay resistance in *Bt* cotton. Significantly heavier boll weight (g) was recorded in *Bt* over *nBt* cotton [24, 29].

Variations found during different planting dates showed significant effect on average boll weight (g) plant^{-1} (Table 2). More average boll weight plant^{-1} was observed in D_1: mid March (3.38) of first field than D_2: mid May (3.07) of second field observation. Normal planted date produced bigger bolls due to higher accumulation of photosynthates and more time was available for boll development and maturity. Prolonged temperature exceeds more than 35º C and less than 25 º C during flowering stage reduces the boll size [30]. Delayed sowing dates encountered with high insect and pest problems at maximum temperature and at low temperature received low solar radiation which resulted less leaf area, growth rate consequently decreases the boll weight and boll number [31, 32].

Number of seeds plant^{1}: Number of seeds plant^{-1} influenced by different cultivars and sowing dates. Significantly highest number of seeds plant^{-1} was recorded in 50% *Bt* cotton (560.44, 476.33) over 50% *nBt* refuge cotton (281.55, 201.77) during different planting dates in two different field observation (Table 2). Variations in results due to different genotypes, more no. of monopodial branches, sympodial branches and more number of bolls was found in *Bt* cotton in comparison to *nBt* cotton. During field experiments, *Bt* cotton proved to be effective against certain target lepidopterous pests whereas in case of non-*Bt* cotton more number of bolls infested with heavy pest problems and affect the final seed cotton yield.

Deviations in temperature of different sowing dates affects significantly number of seeds per plant. Significantly more number of was obtained when sowing in D_1: mid March (511.55) of first field than late sowing during D_2: mid May (402.33) of second field respectively (Table 2). Less number of bolls damaged by bollworms when crop sown in early planting date than late planting date because peak flowering time during may synchronized with most active period for insect pest attack [33].

Weight of lint (g) plant^{-1}: Weight of lint plant was significantly affected due to different cultivar and sowing dates. Significantly maximum weight of lint (g) plant^{-1} was recorded in 50% *Bt* cotton (23.27, 18.79) contrast to 50% *nBt* refuge cotton (11.20, 7.68) in two different fields sowing at different dates. Significant difference found in the fiber quality between different cultivars was prominent [34].
Cotton genotypes vary for fiber length and fiber strength Different planting dates showed significant effect on weight of lint (g) plant in *Bt* cotton. Weight of lint (g) plant^{-1} was found more in D_1: mid March (19.07) date of sowing than delay planting date D_2: mid May (10.75) reduces the weight of lint (g) plant and affects the final yield of cotton [35].

Conclusion
Results indicated the growth and yield parameters was found highest in 50% *Bt* with 50% *nBt* cotton at early sowing date during mid March than late sowing during mid May. When compared 50% *Bt* with 50% *nBt* refuge cotton was found highest in 50% *Bt* than 50% refuge *nBt* cotton except plant height during both sowing dates. No pesticides were applied during the whole crop trail due to presence of *Bt* crops sufficient to protect from bollworm problems but by presence of 50% *nBt* refuge cotton helps to delay resistance against bollworms in *Bt* cotton.

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