



Effect of attractants on activity and abundance of insect pollinators in cotton (*Gossypium hirsutum*)

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ABSTRACT

Cotton (*Gossypium hirsutum* L.) is regarded as partially cross pollinated and largely self pollinated crop; thereby enhancement in the activity of pollinators into the crop during flowering may result into increased quantity and quality of cotton seed. Therefore, the present study was carried out at the experimental farm of ICAR-Central Institute for Cotton Research (CICR), Regional Station, Sirsa, Haryana on a Gangetic alluvial soil during rainy (*khari*) season (April– October) of 2016–17 and 2017–18 under split-plot design keeping genotypes as main plot treatments and spray of 3 attractants, hand pollination and a control as sub-plot treatments to study the activity of various pollinators in cotton and effects of various attractants on abundance of these pollinators. Honey bee, bumble bee, wasp, ants and others pollinators (butterfly, dragon fly, and carpenter bee) were recorded on cotton plant and flowers of different genotypes of cotton. The observations were recorded before and after spray of various attractants for different pollinators. Honey bees were the most abundant recorded species (mean pre-treatment population 0.22–0.23 per plant) of pollinators followed by wasp and others (butterfly, dragon fly and ants) which regularly visited the cotton. The various attractants, i.e. spray of molasses, sugar solution, rose extract @10% concentration, hand pollination and a control (natural pollination occurred) applied thrice during the season did not significantly affect the activity of the pollinators. The highest boll (72 and 63%) and seed setting (83.6 and 84.3%) was recorded in treatment where hand pollination was practised followed by application of molasses to attract the pollinators without any significant effect on yield.

Keywords: Attractants, Bumble bee, Cotton, Diversity, Honey bee, Pollinators, Wasp

Cotton (*Gossypium hirsutum* L.) plant is a shrub and cultivated for its fiber and seeds. It is an angiosperm, dicotyledonous plant which belongs to Malvaceae family. The most widely cultivated cotton species are *Gossypium hirsutum*, *G. herbaceum*, *G. barbadense* and *G. arboreum*. Pollination is the process by which pollen is moved from anthers to the stigma. Cotton is regarded as partially cross pollinated (5–25%) and largely self-pollinated, although introducing pollinators into the crop during flowering has resulted in increased quantity and quality of cotton (Stein *et al.* 2017). Pollen grains of cotton are heavy and sticky in nature; therefore wind is not a factor in the pollination of cotton (Van Deynze *et al.* 2005). Cotton white flowers occur singly, i.e. solitary and each cotton flower is only available for pollination on the first day when flower opens with the corolla and staminal column falling off on the second day (Rhodes 2002). These flowers attract a wide range of insects including bees, flies, butterflies and beetles which

visit flowers to collect nectar and pollen (Green and Jones 1953). Honey bees are naturally attracted by the cotton plant and can travel from miles away to collect its pollen. Visits of bees to cotton plants estimated to increase fiber production by at least 10% in weight while the quantity of seed production and weight of cotton bolls also increases (Pires *et al.* 2014). Around 11–13% more seed setting was recorded by Kuliev (1958) with bee pollination in comparison to seed set without bee assistance in cotton crop. McGregor (1976) reported that in USA, bumble bees are very effective pollinators of cotton pollination. Cotton flowers were visited by 14 species of insect pollinators on both CMS and R line of cotton and contribute 65–70% on CMS and 75–78% on R line (Kumar 2010). Naik (2010) reported that bee pollination helps in increasing boll size, higher number of seeds per boll, lint per boll and other yield parameters. Keeping the above facts under consideration, the diversity of pollinators and effect of various attractants on their abundance was studied.

MATERIALS AND METHODS

The present study was carried out at the experimental farm of ICAR-Central Institute for Cotton Research

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(CICR), Regional Station, Sirsa, Haryana on a Gangetic alluvial soil during rainy (*khariif*) season (April–October) of 2016–17 and 2017–18 under split-plot design keeping four genotypes [i.e. V₁, IC-357197; V₂, IC-359134; V₃, IC-356695 and; V₄, IC-356697] as main plot treatments and five spray of attractants as sub-plot treatments [T₁, Spray of molasses @10% concentration; T₂, Spray of sugar solution @10% concentration; T₃, Spray of rose extract @10% concentration; T₄, Hand pollination and; T₅, Control (natural pollination occurred)] to study the diversity and abundance of pollinators as well as the role of various attractants on the activity of these pollinators in cotton and were replicated thrice. The selection of genotypes for the study was done on basis of their seed setting potential, the germplasm lines of cotton having low seed setting potential, i.e. IC-357197 and IC-359134 as well as high seed setting potential IC-356695 and IC-356697. From each plot, five plants were randomly selected and numbers of various pollinators visiting these flowers and plants in 10 min were recorded between 10 am to 12 noon, when pollinators are most active in flowers and plants at flowering time in cotton plants. The observations were recorded before, after one day and 7th day of application of different attractants (T₁; T₂; and T₃) for different pollinators, in T₄ (managed with hand pollination) and T₅ (control). Three sprays of the various attractants were applied during flowering at 15 days interval during each season to attract the pollinator. Observations were recorded and all the replicated data obtained from this study were statistically analyzed by the *F*-test as per the procedure given by Gomez and Gomez (1984). Least significant difference (LSD) values at *P* = 0.05 were used to determine the significance of differences between treatment means.

RESULTS AND DISCUSSION

Honey bees, bumble bees, wasps, ants and other pollinators (butterfly and carpenter bee) were recorded as regular and frequent pollinators on different genotypes of cotton. During 2016–2017, the number of honey bees visiting the various cotton germplasm lines selected for study, ranged from 0.19–0.28 per plant, wasps ranged from 0.07–0.10, other pollinators ranged from 0.18–0.28, ants ranged from 0.11–0.14 and bumble bees visiting the plant ranged from 0.00–0.07. On 1st day after spray of pollinator attractants recorded highest number of honey bees (0.57 per plant) in germplasm line having low seed setting potential (IC-357197) and lowest number of honey bees (0.51) in germplasm line having higher seed setting potential (IC-356695) but statistically at par with each other. On 7th day after spray maximum number of honey bees (0.19 per plant) recorded in germplasm line with low seed setting potential (IC-359134). On 1st day after spray highest (0.2) and least (0 per plant) number of bumble bees were recorded in germplasm lines IC-356697 and IC-356695, respectively having high seed setting potential. On 7th day after spray, bumble bees visits were negligible. After 1st day of spray, higher number of wasps per plant (0.12)

were recorded in germplasm line having high seed setting potential (IC-356697) and after 7th day of spray, highest population (0.28) was in germplasm line having low seed setting potential (IC-357197). After 1st day spray, higher number of ants (0.32) were recorded in germplasm line having low seed setting potential (IC-357197) and lowest was in germplasm line having high seed setting potential (IC-356697). After 7th days spray, higher number of ants (0.27) were recorded in germplasm line having high seed setting potential (IC-356697). After 1st day spray, higher number of other pollinators (0.16) were recorded in germplasm line having low seed setting potential (IC-359134). After 7th day of spray, higher number of other pollinators (0.25) recorded in germplasm line having low seed setting potential (IC-357197) and (IC-359134) respectively (Table 1). Among the various pollinator attractants applied, the population of honey bees ranged between 0.08–0.25 per plant before treatment and 0.38–0.63 per plant after one day and 0.11–0.33 after 7th day of application. Among the different treatments highest population (0.63) after one day was recorded with the application of T₃ (rose extract @ 10% conc.) but after 7th day of spray, it was maximum (0.33) with the application of T₂ (spray of sugar solution). However, the genotypes as well as attractants could not significantly affect the number of pollinators in cotton. The pollinator recorded in maximum abundance was honey bees followed by wasps (0.01–0.10), ants (0.02–0.23) and others (0.02–0.18) after 7th day of application.

During 2017–2018, the number of honey bees ranged between 0.20–0.47 post spray and highest population was in germplasm line having low seed setting potential (IC-357197). The number of visits of bumble bees were ranged between 0–0.07, wasps ranged between 0.07–0.20, ants 0.07–0.20 and others 0.07–0.33 after spray. Among the various attractants sprayed, the population of honey bees ranged between 0.08–0.41 per plant before treatment and 0.25–0.58 per plant after application of attractants. Among the different attractants, the highest population (0.58 per plant) was recorded in T₃ (rose extract @10% conc.). However, the genotypes as well as attractants could not significantly affect the number of pollinators in cotton. Among the various pollinators, maximum abundance of honey bees was recorded followed by wasps (0.01–0.25), ants (0.00–0.25) and others (0.08–0.42) after application (Table 2).

The data recorded on seed and boll setting (%) efficiency ranged between 62.6–72.0 and 56.2–59.7 during 2016–17 and 2017–18. Similarly seed setting (%) recorded ranged between 72.7–84.8 and 79.8–83.5 during 2016–17 and 2017–18. The germplasm lines having potential of high seed and boll setting (IC-356695 and IC-356697) were recorded with higher seed and boll setting. Whereas among the various treatments applied to attract the pollinators, boll setting (%) ranged between 68–72 and 57–63 during 2016–17 and 2017–18, respectively. Similarly seed setting (%) ranged between 76.3–83.6 and 80.5–84.3 during 2016–17 and 2017–18, respectively. Among the various treatments

Table 1 Average number of pollinators after three sprays during 2016–17

Treatment	Honey bee			Bumble bee			Wasp			Ant			Others		
	Pre spray	1 day	7 day	Pre spray	1 day	7 day	Pre spray	1 day	7 day	Pre spray	1 day	7 day	Pre spray	1 day	7 day
<i>Genotypes/germplasm lines</i>															
V ₁	0.19	0.57	0.16	0.00	0.01	0.00	0.08	0.09	0.28	0.11	0.32	0.03	0.27	0.26	0.25
V ₂	0.23	0.54	0.19	0.00	0.01	0.00	0.08	0.05	0.05	0.16	0.22	0.16	0.22	0.26	0.25
V ₃	0.23	0.51	0.17	0.07	0.00	0.00	0.07	0.06	0.04	0.14	0.18	0.12	0.18	0.24	0.06
V ₄	0.28	0.56	0.13	0.00	0.02	0.00	0.10	0.12	0.05	0.30	0.14	0.04	0.28	0.16	0.02
SEm±	0.06	0.06	0.04	0.01	0.01	0.00	0.03	0.03	0.02	0.06	0.05	0.03	0.03	0.05	0.05
LSD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<i>Pollinator attractants</i>															
T ₁	0.08	0.57	0.24	0.00	0.01	0.00	0.02	0.09	0.10	0.20	0.29	0.02	0.16	0.27	0.02
T ₂	0.26	0.43	0.33	0.01	0.00	0.00	0.06	0.07	0.06	0.16	0.19	0.15	0.27	0.18	0.02
T ₃	0.21	0.63	0.11	0.01	0.01	0.00	0.11	0.11	0.01	0.19	0.17	0.12	0.82	0.17	0.14
T ₄	0.22	0.39	0.17	0.00	0.00	0.00	0.08	0.03	0.01	0.15	0.16	0.09	0.26	0.26	0.13
T ₅	0.25	0.38	0.16	0.00	0.01	0.00	0.09	0.06	0.03	0.19	0.28	0.23	0.31	0.28	0.18
SEm±	0.08	0.09	0.06	0.01	0.01	0.00	0.04	0.04	0.03	0.06	0.16	0.02	0.07	0.08	0.07
LSD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

LSD refers to the least significant difference at 5% probability level.

Treatment details are given under Materials and Methods.

applied to attract the pollinators, highest boll and seed setting was reported in treatments with hand pollination followed by treatment of application of molasses @10% conc., all the treatments except hand pollination were statistically at par (Table 3).

Cotton is generally treated as self-pollinated crop, but cross pollination ranging from 5–50% is also reported (Stephens and Finkner 1953). Insects are important natural pollinators, foraging cotton crop due to the presence of floral nectarines. Sucrose content in the flower nectar especially in CMS flowers affected significantly the honey bee population density and might be a key factor for higher hybrid seed production in CMS lines. The main pollination insect species, namely *Allograpta exotica* (Diptera), *Apis mellifera* have been reported as effective pollinators in cotton agro ecosystems with 12% increase in fiber weight and 17% increase in number of seeds in insect pollinated cotton crops other than conventional cotton (Muhammad *et al.* 2020). The wind is not a factor in the pollination of cotton because the cotton pollen is relatively large, heavy, sticky and watery hence the activity of pollinators are essential (Anonymous 1879). In the present study, spray of molasses @10% conc., spray of sugar solution @10% conc., spray of rose extract @10% conc. were applied as per the earlier reports, because, among various sugar components of the cotton nectar, only sucrose showed highly positive and significant correlation with visiting honey bee frequency (Bertazzini and Forlani 2016). An array of pollinators plays a vital role in pollination of cotton. Ganapathi (2005) concluded that honey bees and other pollinators constituted 89.10 and 10.90%, 83.77 and 16.23% of the total pollinators. In the present study out of different species of pollinators like honey bee, bumble bee, wasp, ant and others (butterfly and blister beetle) were found foraging on Bt cotton blossoms, but, the honey bees were most abundant. Majority of pollinators visited the cotton were hymenopterans as reported by Mc Gregor (1959) and Tanda (1984), who reported that hymenopterans were more abundant than any other pollinators. Among the hymenopterans, honey bees were the most dominant species in the pollination in cotton as compared to other pollinators. El-Sarrag *et al.* (1993) showed that among different hymenopterans pollinators visited, the most dominant species was *A. mellifera* (67.80%) which was followed by *Bombus* spp. (14.70%) and 33% increase in raw cotton yield by cross pollination within the variety with honey bees. The reason for more dominance of honey bees was that they are social insects living in a colony which is not seen in other pollinators. The pollen grains of cotton adhere to the hairs of honey bees collecting nectar and transferred to the stigma of another flower; therefore, these bees are effective agents in pollination of cotton. Occasionally, honey bees will scrape the cotton pollen from their bodies with their legs. The results also suggest that the contribution of a set of different pollinator's species can also be important for cotton production than that of just one species. After honey bee, others insects including butterfly, bumble bee, dragon fly, wasp and ant were also found visiting the cotton

Table 2 Average number of pollinators recorded after three sprays during 2017–2018

Treatment	Honey bee		Bumble bee		Wasp		Ant		Others	
	Pre spray	Post spray (7 th day)	Pre spray	Post spray (7 th day)	Pre spray	Post spray (7 th day)	Pre spray	Post spray (7 th day)	Pre spray	Post spray (7 th day)
<i>Genotypes/Germplasm lines</i>										
V ₁	0.27	0.47	0.00	0.07	0.27	0.20	0.07	0.07	0.00	0.20
V ₂	0.13	0.33	0.07	0.07	0.13	0.13	0.07	0.13	0.07	0.07
V ₃	0.40	0.20	0.13	0.00	0.07	0.07	0.00	0.07	0.00	0.13
V ₄	0.13	0.27	0.00	0.07	0.07	0.20	0.07	0.20	0.00	0.33
SEm±	0.10	0.13	0.06	0.05	0.09	0.11	0.06	0.08	0.03	0.10
LSD (P = 0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<i>Pollinator attractants</i>										
T ₁	0.25	0.25	0.08	0.00	0.17	0.25	0.00	0.25	0.00	0.08
T ₂	0.08	0.25	0.00	0.17	0.08	0.25	0.08	0.17	0.03	0.42
T ₃	0.41	0.58	0.00	0.00	0.17	0.17	0.00	0.08	0.04	0.17
T ₄	0.16	0.50	0.08	0.00	0.17	0.08	0.08	0.08	0.01	0.17
T ₅	0.25	0.25	0.08	0.08	0.08	0.00	0.08	0.00	0.01	0.08
SEm±	0.11	0.15	0.07	0.07	0.10	0.13	0.07	0.09	0.03	0.11
LSD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

LSD refers to the least significant difference at 5% probability level. Treatment details are given under Materials and Methods.

followers, respectively. Any substance that attracts the bees and other pollination insects towards the eliciting source is called attractant. Bee attractants play a beneficial role in enhancing pollination and yield of crops especially when target crop is not so attractive to the bees naturally or when the weather conditions are not conducive for foraging by the bees on target crop. Five types of attractants, i.e. spray of molasses; sugar solution and rose extract @10% conc., hand pollination and control with water were used in study to attract these pollinators in pollination activity in cotton. No significant effects of attractants were observed on the number of pollinator species visiting cotton flowers like wasp, ant and others, except honey bee which show numerical increase in foraging activity of honey bee in cotton, can be attributed to application of attractants or the existing communication behavior in bees. According to Pleasants and Wendel (2010) primary visitors of *Gossypium tomentosum* were introduced species of honey bees and carpenter bees, both of which were pollinating the flowers and no native bee species were seen visiting flowers. The present results corroborate the earlier reports on the efficacy of botanicals as well as pheromone based attractants. Our study also revealed the foraging activity of honeybee increased with spray of pollinator attractants because of presence of specific volatile components of these substances. Though the pollinator attractants applied to enhance visits of pollinators did not exhibited any significant impact on the activity of these pollinators and probably there would have been a beneficial increase in yield of cotton if enhancement in the activity of honey bee and other pollinators may be increased into cotton crop.

Table 3 Boll and seed setting (%) in germplasm lines and different pollinator attractants

	2016-17		2017-18	
	Boll setting (%)	Seed setting (%)	Boll setting (%)	Seed setting (%)
<i>Genotype</i>				
V ₁	62.6	72.7	56.2	81.5
V ₂	65.4	73.3	56.3	79.8
V ₃	68.6	80.8	59.7	83.0
V ₄	72.0	84.8	58.6	83.5
LSD (P = 0.05)	1.50	3.40	5.60	3.00
<i>Treatment</i>				
T ₁	70.2	79.5	60.7	82.8
T ₂	69.0	78.5	59.0	82.0
T ₃	68.2	77.4	58.4	81.5
T ₄	72.0	83.6	63.0	84.3
T ₅	68.0	76.3	57.0	80.5
LSD (P = 0.05)	2.40	3.80	2.20	1.70

LSD refers to the least significant difference at 5% probability level. Treatment details are given under Materials and Methods.

Among all pollinators recorded honey bee (*Apis mellifera* and *A. florea*), bumble bee, wasp, ant and others pollinators (butterfly and carpenter bee) on cotton plant and flowers of different germplasm lines of cotton, honeybees were the most dominant species followed by wasp and others (butterfly, dragon fly and ants) which regularly visited the cotton. Although cotton is self pollinated and

partially cross pollinated, but to enhance the activity of the pollinators various attractant applied, i.e. spray of molasses, sugar solution, rose extract @10% concentration, applied thrice during the season did not significantly affect the activity of the these pollinators in comparison to hand pollination and a control. However, hand pollination reported to be superior method to improve boll and seed setting percentage.

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