POLLINATORS DIVERSITY AND THEIR EFFECTS ON RAPESEED (*Brassica campestris L. var. toria*) PRODUCTION AND PRODUCTIVITY IN CHITWAN, NEPAL

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ABSTRACT

Crop pollination is crucial for increasing yield, ensuring food security and improving livelihoods. To quantify the response of pollinators on rapeseed, an experiment was conducted in randomized complete block design (RCBD) at three agro-ecological sites: Semi-natural (Megauli), organic (Fulbari) and intensive agriculture (Jutpani) Village Development Committees of Chitwan district in 2012/013 and 20013/014. The treatments were: i) open pollination; ii) plants caged with honeybees (Apis melifera L.*); iii) hand pollination; and iv) control (plots caged without pollinators) replicated four times. Pollinators visiting rapeseed flowers, plant height, branch number, siliqua/plant, pods weight/ siliqua, test weight, and seed yield/hectare were recorded. The dominant pollinators were Hymenopterans mostly honeybees. The impact of pollinators on each system resulted in significantly increased yield attributes compared to no-pollination, which clearly indicates the need of integrating managed pollination and pollinators' conservation to sustain rapeseed production in Chitwan through biodiversity-based ecosystem services.*

Key words: Honeybees; Intensive agriculture; Open pollination; Pollinators; Rapeseed; Semi-natural;

INTRODUCTION

Oilseed is the dominant winter season cash crop of Nepal. It is mostly grown after monsoon maize in upland and after early rice in lowland of Terai, inner Terai and mid-hills of Nepal. Oilseed occupied 213706 ha and produced 176186mt in 2010/11, and its area and production increased to 214835 ha and 179145mt, respectively in 2011/2012 (MoAC, 2011/2012). In oilseeds, rapeseed (*Brassica campestris* L. var. *toria*) alone occupies about 85% of the area in the country (Basnet, 2005). However, its productivity is low, i.e. 0.83 mt/ha. Due to self incompatibility it requires sufficient pollinating agents for better pollination and seed production.Over 80% of pollination is performed by insects, and among insects bees contribute nearly the same (Kevan and Phillip, 2001; Kwapong, 2007).

Honeybees visit rapeseed flowers for collection of both pollen and nectar, which in turn results into cross-pollination of florets (Sharma *et al.*, 2004). Thus, the main significance of honeybees in beekeeping is pollination, whereas the hive products (honey, wax etc.) are of secondary value (Verma, 1990). Studies have shown that insect pollination increased pollen deposition in canola/rapeseed crops leading to increased fruit set and seed production per plant, and decreased the variance of seed sets, and also enhanced better quality,uniform ripening and plant vigor (Winston 2005; Thapa, 2006; Garibaldi *et al.*, 2010; Klien *et al.*, 2007). Rapeseed pollination by

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honeybees *Apis cerana* F. and *Apis mellifera* L increased oil content by 3.17% and 1.44%, respectively over open pollination and by 6.86% and 5.07% over caged plants (Dhakal, 2003).

Evidences have shown that low pollinator abundance and diversity have been appearing in different parts of the world (Kasina *et al.*, 2009). In Nepal, it was reported that after heavy use of chemical pesticides all domesticated bees were wiped out in Ilam and Nuwakot, and also many colonies were destroyed in Chitwan (Sharma, 1994; Thapa, 1994).

In this context, the efforts were directed to improve crop yield by applying other production inputs rather than pollination but did not bring desired results without the use of honeybees to enhance the productivity of different cultivated crops (Verma, 1992). The manmade agro-ecosystem exerted pressure and forced to decline pollinators and their diversity, which resulted in reduced agricultural productivity again threatening biodiversity (Thapa, 2006). Current evidence suggests pollinators need to be conserved and managed sustainably. It also requires long-term studies to assess the stability of pollination service for each crop because the pollinating insects are known to show high temporal variation as well as changes brought about by habitat changes (API, 2006). Therefore,this study was conducted to assess pollination deficit in rapeseed crops under farmers' managed agricultural systems, which could be useful in formulating strategies to improve utilization of pollination for crop production.

MATERIALS AND METHODS

RESEARCH SITE AND RESEARCH DURATION

The research was conducted in three sites, i.e. very close to natural habitat (Meghauli), organic farming (Fulbari), and intensive agriculture practiced site (Jutpani), of Chitwan district for two years (2012/013 and 2013/014).The experiment was laid out in randomized complete block design (RCBD) with four treatments: i) open pollination; ii) hand pollination; iii) bee supplementation with *Apis melifera* L.; and iv) control (without pollination) replicated four times in each agro-ecosystem. The plot size of each treatment was 3 m x 5 m (15m^2). Two framed broods of *A. mellifera* L. were evaluated in bee supplemented plots). In case of hand pollination, the treatment was evaluated in 1 m^2 area. When the crop started anthesis (during October), honeybee colonies were placed on the respective experimental plots caged by mosquito nets (5m x 3m x 1.5 m) starting from initial to cessation of flowering. For assessing the pollinators' diversity and intensity, a standard plot of 25m x 50m was also selected at farmers' fields (FAO, 2011).

Rapeseed (variety: Pragati) was broadcasted on 10 November, 2012 and 30 October, 2013 @ 6 kg/ha in well pulverized soil. The fertilizers applied were FYM @20 mt/ha, NPK @ 60:60:40 kg/ha and Sulphur @30 kg/ha, respectively. Full FYM, half nitrogen, full phosphorus and potassium were applied as a basal dose and remaining nitrogen as top dressing at 25 days after sowing (DAS) and other cultural practices followed as per improved cultivation practices.

The weight of 1000 seeds, number of seeds per siliqua was assessed by randomly sampling five plants per plots. From each plant, 20 pods were collected starting from the tip of the main inflorescence and the number of seeds in each pod was counted. Insect visits were observed and recorded at 10%, peak and 10% remaining flowers by counting the number of flower visitors per m^2 for five minutes. The observations were made at 10-11 am under sunny weatherconditions with temperature above 17°C. Collected data were analyzed in computer using Excel and MSTATC software programs.

RESULTS

POLLINATOR ABUNDANCE AND DIVERSITY

Hymenopteran pollinators appeared in greater number in all sites during the both years. The relative abundance of pollinators in different sites during two years is shown in Table 1. It shows that the mean population of Diptera, Lepidoptera, Coleoptera and other insects groups were higher in intensive agriculture sites in 2012/013. The population of flies was the highest in year 2013/014 in intensive agriculture site followed by Coleopteran, Lepidopteran insects, respectively. However, higher numbers of Coleopteran and Lepidopteran flower visitors were crop pests in intensive agriculture field, which were resistant pests due to massive use of pesticides.

Table 1.Abundance of insect pollinators in rapeseed field of different agro-ecosystems in 2012/13 and 2013/14 at 10%, peak and 10% remaining to flowering

	Semi-natural					Organic					Intensive Ag.				
	10%F *	Peak F*	10%R F*	Total	%	10%F	Peak F	10%R F	Total	%	10%F	Peak F	10%R F	Total	%
Year 2012/013															
Hymenoptera	55.8	83.0	55.8	194.6	56.2	71.0	70.9	54.2	196.1	55.9	28.0	23.0	30.0	81.0	35.1
Diptera	20.0	30.3	20.3	70.6	20.4	24.0	28.7	19.0	72.1	20.6	13.0	8.8	17.0	38.8	22.1
Coleoptera	14.0	16.3	13.5	43.8	12.7	13.0	15.0	13.0	41.3	11.6	12.0	11.0	8.0	31.0	16.2
Lepidoptera	6.7	8.5	7.0	22.2	6.4	7.0	8.2	9.5	24.7	7.0	4.3	13.0	4.8	22.1	22.5
Hemiptera	1.7	3.0	2.8	7.5	2.2	3.2	2.8	2.2	8.2	2.3	2.2	1.2	1.3	4.7	1.8
Orthosptera	2.3	2.7	2.5	7.5	2.2	2.5	2.7	3.8	9.0	2.6	2.2	1.7	2.3	6.2	2.3
Total				346.2	100				351.4	100				183.8	100
Year 2013/014															
Hymenopera	47.3	58.8	55.3	161.4	64.8	54.0	75.8	45.0	175.1	63.7	52.0	39.0	34.0	125.0	39.9
Diptera	8.8	22.4	14.0	45.2	18.1	12.0	13.2	8.8	34.1	13.8	20.0	16.3	9.1	45.0	41.9
Coleoptera	14.2	25.2	23.8	63.2	25.4	19.0	17.8	13.0	49.3	19.9	11.0	11.0	7.0	29.0	7.9
Lepidoptera	9.0	7.0	10.2	26.2	10.5	12.0	10.7	12.0	34.7	14.2	11.0	9.7	14.0	34.7	11.3
Hemiptera	1.7	2.3	3.0	7.0	2.8	4.7	2.3	2.7	9.7	3.9	4.7	2.8	3.5	11.0	5.2
Orthoptera	1.2	2.7	5.5	9.4	3.7	3.8	5.0	1.8	10.6	4.3	1.5	2.2	1.2	4.9	1.7
Total				312.4	100				313.5	100				249.6	100

*10% F = 10% flowering, Peak F= peak flowering, 10%RF = 10% remaining of flowering

Abundance of pollinators at different agro-ecosystems: Different types of pollinators visiting in rapeseed field were: Hymenopterans (*A. cerana, A. melifera, A. dorsata, A. florea*, *Andrena* sp, *Xylocopa* sp, *Bombus* sp, and other wasps), Diptera (*Syrphus* sp, Eristalis sp, *Muscus* sp and *Tachinid* sp), Lepidoptera (*Pieris brassicae nepalensis*, *Pelopidas* sp, *Lampides boeticus*, *Nyctemers streama*) and Coleoptera (*Coccinella* sp, *Aulacophora fobicolis* and *Mylabri*s sp). In general, Hymenopterans were the major pollinators found in all agro-ecosystems (Figures 1, 2).

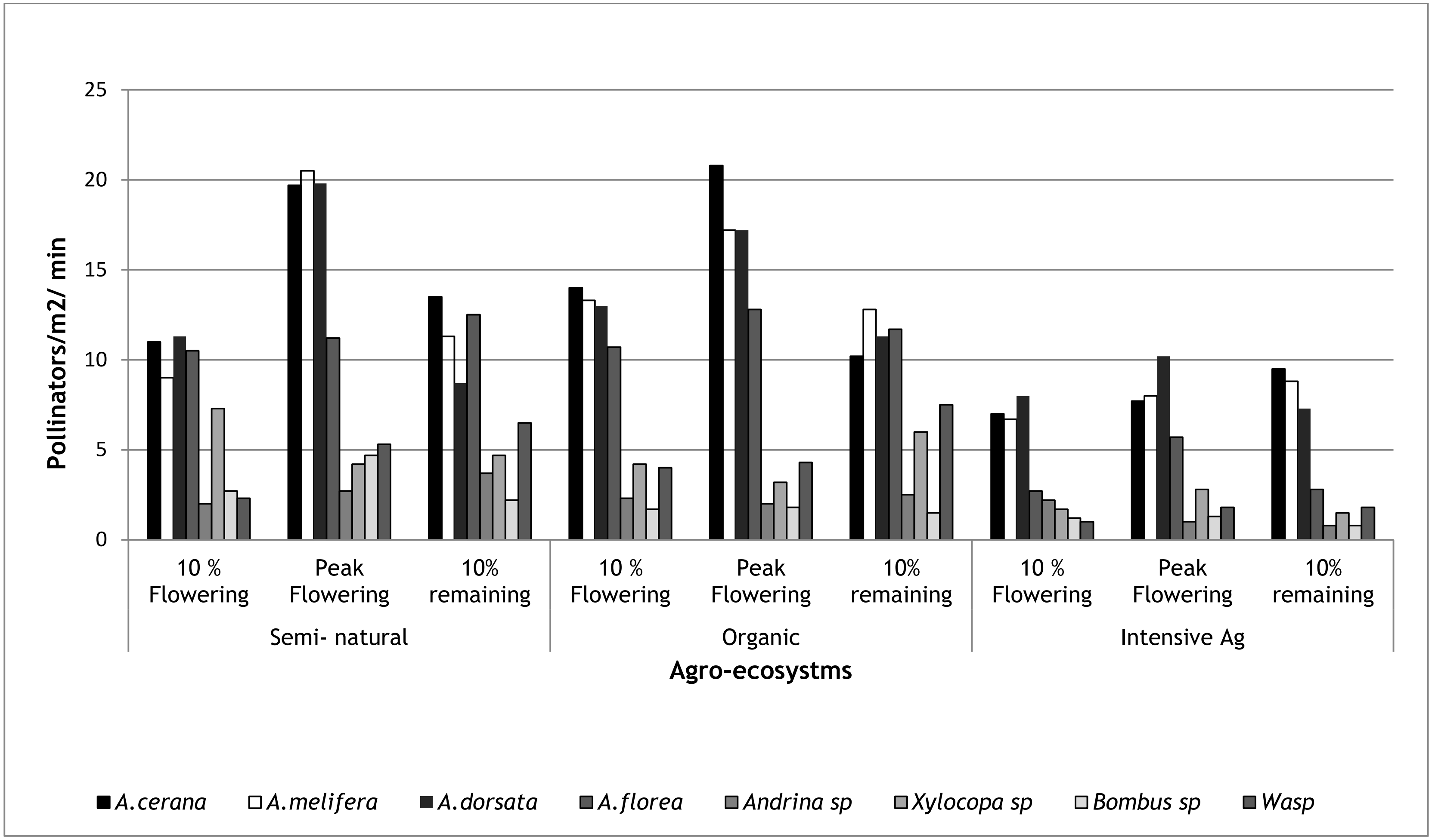

Figure 1. Abundance of insect pollinators in semi-natural, organic and intensive rapeseed fields of Chitwan at 10%, peak and 10% remaining of flowering in 2012/013

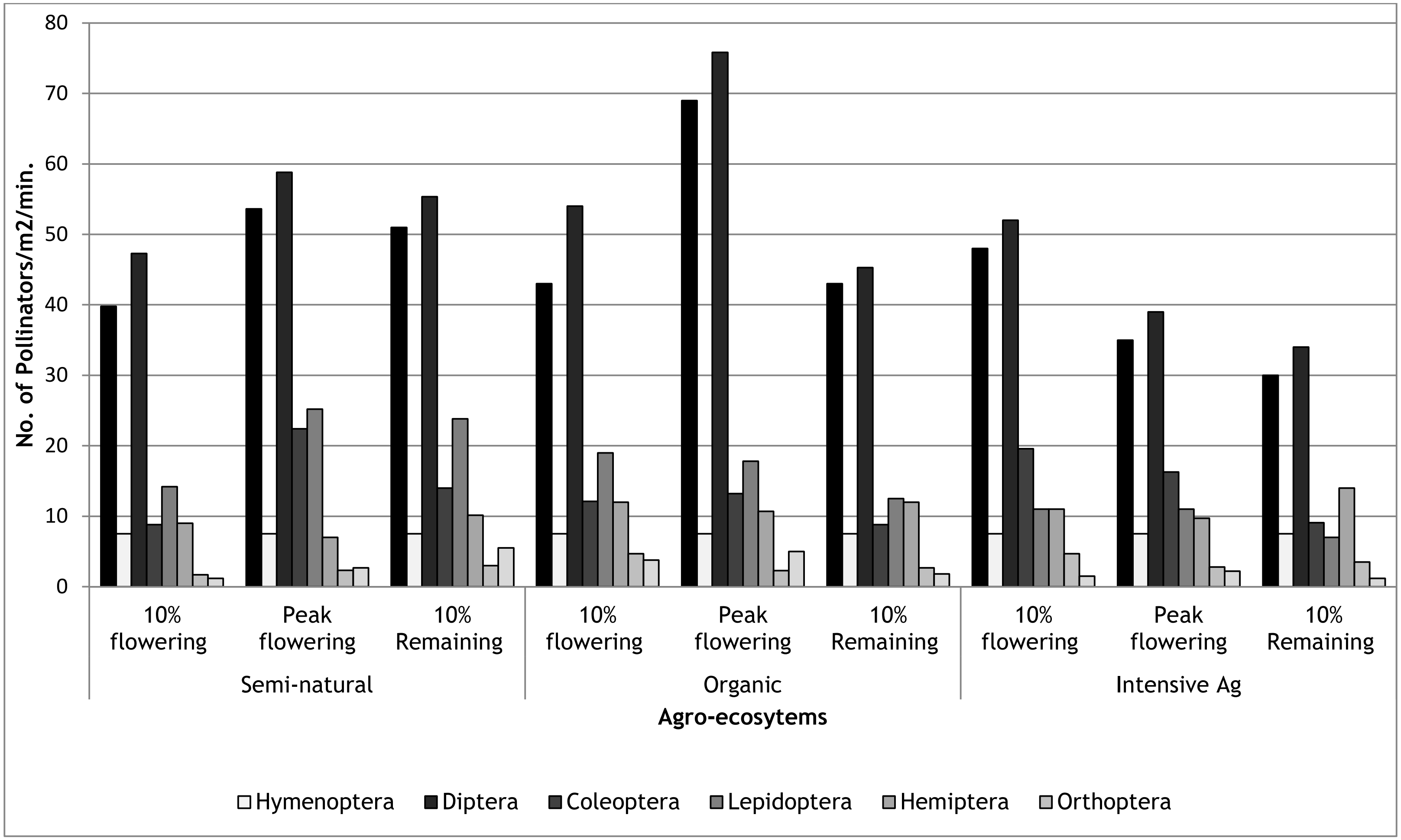

Figure 2. Abundance of insect pollinators in semi-natural, organic and intensive rapeseed fields of Chitwan at 10%, peak and 10% remaining of flowering in 2013/014

Figure 1 shows that Hymenopteran pollinators were higher in number followed by Diptera, Coleoptera, Lepidoptera, Hemiptera and Orthoptera in 2012/013. Increasing number of pollinators was observed in the year 2013/014 with similar trends and dominancy of Hymenoptera (mostly bees) also in 2013/014. The pollinators were found in higher numbers in semi- natural and organic sites as compared to intensive agriculture practiced site during the both years. The lower number of pollinators in intensive agriculture system might be due to commercial farming (higher application of chemicals, hybrid varieties), low organic matter incorporation in the soil and the distance from the natural habitats of pollinators.

EFFECT OF POLLINATION IN PRODUCTION

The treatments in each agro-ecosystems resulted in significant differences in observed characteristics. The effect of pollinators on yield attributes is shown in Tables 1 and 2.

Table 1: Effect of treatments on rapeseed plant height and number of branches under different agro-ecosystems during rapeseed growing seasons of 20 12/013 and 2013/014[1]

Agro-ecosystem	Treatment	Plant height (cm) †		No. of branch†	
		2012/013	2013/014	2012/013	2013/014
Semi-natural	Open pollination	66.5^{b}	69.8^{c}	4.8^{c}	4.3^{c}
	Bee supplementation	70.9^{b}	70.6^{b}	4.5^{c}	4.8^{bc}
	Hand pollination	76.2^{a}	81.7^{a}	6.0^{b}	5.8^{b}
	Control	77.3^{a}	83.2^{a}	7.3^{a}	7.3^{a}
Organic	Open pollination	67.7^{c}	68.9^{d}	4.5^{c}	3.3^{c}
	Bee supplementation	71.0^{bc}	74.8^{c}	6.3^{b}	4.3^{b}
	Hand pollination	75.5^{b}	80.3^{b}	6.3^{b}	4.3^{b}
	Control	83.6^{a}	87.9^{a}	7.0^{a}	7.0^{a}
Intensive-Ag	Open pollination	67.9^{a}	69.2^{b}	4.8^{b}	3.5^{c}
	Bee supplementation	71.2^{a}	68.9^{b}	4.5^{b}	4.3^{b}
	Hand pollination	77.9^{b}	84.3^{a}	6.3^{a}	4.3^{b}
	Control	86.5^{a}	86.3^{a}	7.5^{a}	6.5^{a}
	F-value	*	*	*	*
	LSD value (≤0.05%)	5.1	5.1	1.022	1.022
	CV%	4.75	4.75	13.44	4.75

[1]Randomized Complete Block Design Combined over locations and years

† Means followed by the same letter in each column are not significantly different by DMRT at≤ 0.5 level

In 2012/013, the effects of pollination treatments on plant height varied significantly in each agro-ecosystem, i.e. plant height in open pollination, bee supplementation and hand pollination plots were significantly lower as compared to the control at each agro-ecosystem. Similarly, the plant height was significantly lower in pollination treatments than in the control in the year 2013/014 too.

The highest plant heights were recorded on control plots. The branch number was lower in semi-natural and organic sites as compared to control at each site.

Table 2: Effect of treatments on the seeds/siliqua, 1000 grains weight and seed yield in different agro-ecosystems in 2012/013 and 2013/014[1]

Agro-ecosystem	Treatment	Siliqua/ plant†	Seeds/ Siliqua†			1000 grain weight (g)†		Seed yield (mt/ha) †	
		2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014
Semi-natural	Open pollination	49.8^{b}	58.5^{a}	12.5^{a}	11.3^{a}	2.875^{a}	3.063^{a}	1.254^{a}	1.097^{a}
	Bee supplementation	61.3^{a}	62.0^{a}	12.8^{a}	13.0^{a}	2.625^{ab}	2.947^{ab}	0.993^{ab}	1.093^{a}
	Hand pollination	46.5^{b}	45.3^{c}	12.3^{a}	11.5^{a}	2.2^{bc}	2.475^{b}	0.823^{b}	0.861^{a}
	Control	49.3^{b}	51.8^{b}	4.8^{b}	7.8^{b}	1.975^{c}	1.775^{c}	0.447^{c}	0.479^{b}
Organic	Open pollination	50.5^{b}	56.5^{a}	11.5^{a}	11.3^{a}	3^{a}	2.9^{a}	1.184^{a}	1.226^{a}
	Bee supplementation	58.3^{a}	60.0^{a}	13.5^{a}	13.0^{a}	2.85^{a}	2.7^{a}	0.819^{b}	1.091^{a}
	Hand pollination	42.5c	46.3^{c}	12.8^{a}	12.8^{a}	2.275^{bc}	2.525^{a}	0.754^{bc}	0.926^{a}
	Control	49.3^{bc}	51.8^{Ab}	5.5^{b}	7.8^{b}	2^{c}	1.65^{b}	0.51^{c}	0.488^{b}
Intensive-Ag	Open pollination	47.3^{b}	47.8^{b}	9.8^{b}	11.0^{b}	2.532^{a}	2.625^{a}	0.898^{b}	0.907^{a}
	bee supplementation	61.3^{a}	61.3^{a}	13.8^{a}	14.3^{a}	2.675^{a}	2.625^{a}	1.081^{a}	1.188^{a}
	Hand pollination	46.3^{b}	47.5^{b}	13^{a}	13.5^{a}	2.2^{ab}	2.4^{ab}	0.829^{b}	0.951^{a}
	Control	35.3^{c}	35.5^{c}	4.8^{c}	9..3^{b}	2.1^{b}	1.775^{b}	0.535^{c}	0.476^{b}
	F -value	*	*	*	*	*	*	*	*
	LSD (≤0.5%)	6.88	6.88	2.436	2.436	1.78	1.78	0.5456	0.5456
	CV%	21.13	21.13	11.64	11.64	11.42	11.42	15.72	15.72

[1] Randomized Complete Block Design combined over locations and years.

† Means followed by the same letter in each column are not significantly different by DMRT at≤ 0.5 level

Pollination treatments showed significantly increased number of seeds/siliqua, 1000 grain weight and grain yield as compared to control. Number of siliqua per plant did not differ in all locations. Seeds weight was higher in semi-natural and organic fields with higher number of seeds/siliqua at all sites and test weight was significantly higher in open pollinated plots. In the case of grain yield, open pollination and bee supplementation treatments resulted in increased yield as compared to hand pollination. In each site, grain yield was significantly higher in pollinated treatments as compared to the control. Hence, in each agro-ecosystem, the role of pollination on yield attributers was significantly justified.

DISCUSSSION

Rapeseed field harbored many species of bees that collected nectar and pollen. Even though there were no hives near the rapeseed field, there were honeybees. The open field attracted bees in higher number, which were beneficial for both nectar and pollen collectors. The semi- natural and organic sites harbored greater number of bee pollinators than the intensive agriculture site due to the least disturbance upon the environment and suitable nesting places. Hence, there was no necessity to supplement bee hives for pollination purpose in the semi-natural and organic sites. However, the main pollinators, i.e. honeybees and solitary bees were low in intensive agriculture which needed supplementation to increase pollination as well as production of crops.

The effect of pollinators on yield of rapeseed depends on the density of the pollinators. This study showed the potential yield improvement that can exist in semi-natural or organic or intensive agricultural agro-ecosystems due to presence of pollinators. In Quebec, improvement in seed yield upto 46% was in the presence of three honeybee hives per hectare as compared to no bee hives (Sabbahi *et al*., 2005) in rapeseed. In another research in oilseed rape (*B. napus*), there was 13% seed yield increase in plots with bees as compared to those without bees (Abrol, 2007). The low production of rapeseed in the intensive agriculture sites might be due to lower numbers of bee populations because this site was four km. away from the natural forest and shrub areas. Regression analysis predicted by Manning and Boland (2000) indicated that the number of pods/plant decreased @15.3 pods/plant (equivalent to a 16% loss) as distance increased 1000 m from an apiary. Shortening the blooming periods by 3-5 days in the research observed in semi-natural and organic field is similar to Abrol (2007) that pollinators can contribute to bring uniformity and early pod setting, which reduce blooming period of Argentine canola (*B. napus*) by 3.8 days, with reduced number of flowers that the plant had to produce to reach its carrying capacity (Sabbahi *et al*., 2006). Simon Fraser University in British Columbia found that bee abundance was the greatest in canola fields that had more uncultivated land within 750 m of field edges and seed set was greater in fields with higher bee abundance (Morandin and Winston, 2006); this could be the reason for higher yield in semi-natural fields. Considerable yield in intensive agriculture was obtained in the condition of low population of bees which could be supported by the availability of dipterans population especially syrphid flies, because study has showed that some species of hover flies (Syrphidae) also significantly increased seed set and yield in canola (Jauker and Wolters, 2008).

Research conducted in different parts of world shows that in natural or undisturbed land or in organic land, there was conservation of pollinators with higher densities and diversities. The risk seen everywhere is insecticide applications that reduced pollinators' abundance in the field. Diversity was related to the number of flowering plants and insect pollinators, which increased the number of pods, seeds per pod, seed weights per plant, and seed germination (Atmowidi *et al*., 2007). This research is concurrence with the view of Gallai *et al*. (2009), and Ricketts (2004) that the decrease in honeybee populations (lack of native pollinators) can cause losses in productivity in intensive agriculture site as the higher number of bee abundance and diversity were reported in semi-natural habitats than agricultural fields (Mackenzie and Winston, 1984; Banaszak, 1996; Calabuig, 2001). The variation in pollinators in this study was similar to Moradin (2005) that about 30, 23 and 40% variation in bee abundance was recorded in organic, conventional and GM fields, respectively. According to finding of Osborne *et al*. (1991) and Svensoon *et al*. (2000), availability of nesting sites for wild bees created favorable habitat for bees on semi-natural land as compared to agriculture land. The population of wild bees is declining in agricultural areas due to habitat loss, which is finding of Allen-Wardell *et al*. (1998). Undisturbed landscapes would increase the likelihood of solitary bee pollinator services in agricultural landscape (Morandin *et al*., 2007) which acts as a proxy for increasing wild pollinator densities (Melathopoulus *et al*., 2015). A recent study has demonstrated, for instance, that the benefit to crop yield from animal-mediated pollination depends on pest control levels (Lundin *et al*., 2013). However, pollinator-mediated yield is strongly conditioned by cultivar and their spatial arrangements in fields (Bell*et al*., 2012; Klatt *et al*., 2014), as well as environmental conditions and farm management practices (Boreux *et al*., 2013; Groeneveld *et al*., 2010; Hoover *et al*., 2012; Lundin *et al*., 2013; Melathopoulos *et al*., 2014).

CONCLUSIONS

Rapeseed (*Brassica campestris* L. var. *toria*) which was not pollinated by bees resulted in taller plants height, higher branch numbers and lower yield. Plants that were pollinated by bees reduced flowers longevity and increased seed number per pod, and test weight ultimately producing higher seed yield. The numbers of pods/plant and yield were found to be significantly different among the treatments, i.e. with and without bees ($P \leq 0.05$). The impact of pollinators on each system has resulted in significantly increased yields as compared to restricted pollination. There is deficit in pollination in intensive agriculture field, which has greatly reduced rapeseed yield. Hence, the suitable hibernating places, availability of pollen and nectar source, less human intervention and less chemical pesticide resulted in the higher population of visitors among the flowers. Integrating conservation and suitable management of pollinators is therefore crucial to sustain agriculture productions through optimized management of agronomic inputs and biodiversity-based ecosystem services.

While considering the pollination management, rational decision of growers includes cultivation and maintenance of “bee pastures”, consisting of diverse native or non-native flower-rich plantings maintained in fallow areas, field margins, and conservation of buffer strips to conserve bee pollinators. Hence, it is necessary to take steps to improve ecosystem service (by pollinator) properly for the betterment of people as it leads to sustainable production and food security.

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