

Full Length Research Paper

Effect of Eco-friendly formulations on foraging behaviour of Honeybee, *Apis mellifera* (L.) in Mustard crop-A novel approach

Brijesh Bisht^{1*} and Ruchira Tiwari²

^{1*}-Ph.D Scholar, Department of Entomology, College of Agriculture, G. B. Pant University of Agriculture and Technology Pantnagar, Pin No: 263145, Distt. Udham Singh Nagar (Uttarakhand) INDIA.

²-Assistant Professor, Department of Entomology, College of Agriculture, G. B. Pant University of Agriculture and Technology Pantnagar, Pin No: 263145, Distt. Udham Singh Nagar (Uttarakhand) INDIA.

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Corresponding Author:

Brijesh Bisht

Ph.D Scholar,
Department of
Entomology, College of
Agriculture, G. B. Pant
University of Agriculture
and Technology
Pantnagar, Pin No:
263145, Distt. Udham
Singh Nagar
(Uttarakhand) INDIA.

Abstract

Indian mustard (*Brassica juncea* (L.) Czernj. Cosson), is an excellent source of vegetable oil. Being cross pollinated crop attracts large number of pollinators, among them, honeybees being the most prominent. To analyse the foraging activity i.e. foraging rate and time of honeybee, *Apis mellifera* on mustard crop treated with eco-friendly formulations, a novel study was conducted at G.B.P.U.A & T Pantnagar, Uttarakhand during rabi crop season 2015-16. The eco-friendly formulations included animal urine collected from different cow breeds, buffalo and goat @ 20% along with cow urine based plant extracts i.e. Neem leaf cow urine extract (NLCUE) and Ajwain seed cow urine extract (ASCUE) @ 1% and 2% were sprayed during peak bloom period of mustard crop. The data was collected on the foraging rate and speed of forager honeybee on the flowers of treated and untreated mustard crop at different time intervals. The results showed that there was no adverse effect of tested eco-friendly formulations on foraging behaviour of honeybees, and the maximum foraging rate was observed during the noon hours i.e. 12:00 to 01:00 PM with maximum foraging rate on mustard crop sprayed with desi cow urine @ 20% with (24.0 flowers/bee/min), while the minimum foraging rate was recorded in crop sprayed with ASCUE @ 2% (06.67 flowers/bee/min). The foraging speed was recorded maximum on crop sprayed with holstein cow urine @ 20% (5.0 sec/bee/flower) and minimum in goat urine @ 20% (2.0sec/bee/flower) whereas, the foraging rate in untreated mustard crop ranged from (15.0-7.0 flowers/bee/min.). Thus, it may be concluded that in this novel research work which was conducted for the first time worldwide and can easily be incorporated to enhance the pollination services of honeybees in mustard crop.

Key words: Cow urine, Eco-friendly, Foraging behavior, Honeybee, Mustard

Introduction

Indian Mustard (*Brassica juncea*) belongs to family *Brassicaceae* or *Cruciferae* commonly known as 'rapeseed mustard' is one of the most important source of vegetable oil worldwide. India is the largest rapeseed growing country in terms of area occupying (20.2%) area and second in terms of production (10.7%) after China (Singh *et al.*, 2014). It is a rabi season crop and generally the sowing of mustard crop starts from mid-September continues up to last week of October for timely sowing under North Indian conditions. Mustard is a rich source of bee flora and provide them with both pollen and nectar as a sweet reward for pollination and in turn gets pollinated. Masierowska, 2003, reported that the total amount of nectar secreted per 100 flowers of *Brassica juncea* range 11.76 to 165mg, averaging 79.85mg and sugar in nectar was high enough to attract pollinators upto 52%. Honeybees play an important role as a carrier of pollen from anther to stigma as it is necessary for cross pollinated crops.

Honeybees are specialized for pollination service as their body parts are morphologically adapted for it i.e. presence of corbicula or pollen basket in hind legs to carry loads of pollen. Honeybees along with other pollinators present in the ecosystem provide free service in the ecosystem to pollinate the crops and around 70% pollination is carried out by bees alone (Klein *et al.*, 2007). The yield of brassica crop depends upon the effective visitation rate of honeybee (Bhalla *et al.*, 1983). Even the role of honeybee in elevating the pollination and subsequently increasing the yield of self compatible crop (*Brassica napus*) has been studied by (Rosa *et al.*, 2009). The maximum foraging rate and time of *Apis mellifera* under caged and open pollination conditions on mustard crop was found 6.24bee/1m²/2min and 2.33sec/bee/flower, respectively (Kunjwal *et al.*, 2014).

Besides, pollinating the crop the honeybees have to face the danger of exposure to toxic chemicals and that result in their mortality or contaminating the bee hive with toxic residues of chemicals on crop. (Melisie *et al.*, 2015) in studied the effect of different

chemicals on honey bee (*Apis mellifera*) under laboratory conditions and found that the most of the insecticides result in bee mortality within 24hr of exposure. However, the dependency of chemicals on mustard crop can be reduced by using eco-friendly formulations that can serve the basis of plant protection. Gupta, 2005 reported that incidence of mustard aphid can be safely managed by 3-4 foliar applications of neem kernel extract in cow urine (3%) alone which was comparable to synthetic chemical, dimethoate 0.03% in mustard crop. Neem also have got many potential ingredients such as Azadirachtin and limonene which not only provide protection against diseases but also ward of insect pest by acting as ovipositional and feeding deterrent as proposed by (Singh, 2011). As far the effect of eco-friendly formulations on honey bees is considered, the use of cow urine has been considered as Sanjivani for honeybee disease management with no adverse effect on bees as reported by (Tiwari, 2015).

Thus, a trial was conducted in the Norman Borlaug Crop Research Centre (NBCRC) G.B. Pant University of Agriculture and Technology Pantnagar, Uttarakhand in 2015-16 to evaluate the effect of these eco-friendly formulations on foraging behaviour *i.e.* foraging rate and time of *Apis mellifera* in mustard crop.

Materials and Method

Time of Sowing and Field Layout

The experiment was conducted using Mustard (*Brassica juncea*) variety Varuna T-59, which was sown on last week of September during rabi crop season 2015-16 at NBCRC, G.B. Pant University of Agriculture and Technology Pantnagar, Uttarakhand, to study the effect eco-friendly formulations on foraging behaviour of *Apis mellifera* in mustard crop. The experiments were conducted following Randomized block design with 3 replications each of eco-friendly formulations sprayed on the mustard crop. All agronomic parameters were considered and practised during the cultivation of crop. The plant to plant and row to row spacing was taken as 20 and 30cm, respectively. The field was divided into 30 experimental plots having area of 3x4m² each with total area 3600m².

The tested eco-friendly formulations used in the experiments were generally comprised of animal urine collected from Cow breeds *i.e.* (desi, holstein and jersy), buffalo and goat which were collected from nearby dairy farm and from houses of local farmers. The animal urine used @ 20% for the experiments used was prepared by dissolving 200ml of animal urine in 800ml of water. Accordingly, cow urine based neem leaves and ajwain seeds extracts were prepared @ 1% and 2% each in 20% solution of desi cow urine by taking 10g and 20g of neem leaf powder / ajwain seed powder separately and mixing in 990ml and 980ml of 20% of desi cow urine, respectively, and kept for 3-4 days for fermentation and finally the fermented extracts were thoroughly strained with the help of muslin cloth and used for spraying on the mustard crop and formulations were sprayed after calculating the spray solution on the basis of 500 liter/ha.

Data Collection

The effect of various eco-friendly formulations on the foraging behaviour of *Apis mellifera* recorded at the full bloom period of mustard crop by collecting data on the foraging rate which was recorded by the number of flowers visited by a single forager (honeybee) per minute while foraging speed of forager honeybees was calculated by noting down the time spent by a forager on single flower for collecting pollen or nectar. For proper foraging activity of honeybees, one beehive containing 10 frames full of honeybees was placed near the experimental field of mustard crop by facing hive entrance eastwards. The sprays of eco-friendly formulations were done twice at interval of 15 days during the evening hours and data was collected on the next day on foraging rate and foraging speed of honeybees on the treated and untreated mustard crop. The rate and time of foraging of bees was recorded at three different time intervals of the day *i.e.* first at 9:00-10:00 AM in the morning followed by 12:00-1:00 PM in the noon and lastly at 4:00-5:00 PM in the evening. The data collected from the experiments were subjected to statistical analysis using Randomized Block Design.

Results

The foraging rate of honeybee, *Apis mellifera* showed great variability in different time intervals, where the maximum foraging rate *i.e.* honeybees visit to mustard flowers (24 flowers/bee/ min) was observed during the noon time *i.e.* between at 12:00-1:00 PM on the mustard crop sprayed with desi cow urine @ 20% , while the minimum visits of 6.67 flowers/ bee/min. was recorded in (*Trachyspermum ammi*) Ajwain Seed Cow urine Extract (ASCUE) @ 2% between 3:00-4:00PM. On the analysis of foraging rate of honeybees at different time intervals, the maximum visits by forager bees was recorded at 9:00-10:00AM in mustard crop sprayed with holstein cow urine @ 20% *i.e.* (18.66 flowers/bee/min), followed by (15.0 flowers/bee/min) in jersy cow urine @ 20%, while the visits of forager bees in desi cow urine, buffalo urine and goat urine @ 20% was comparable at (13.0 flowers/bee/min). The minimum foraging rate of honeybees was observed at 9:00-10:00 AM in Neem Leaf Cow Urine Extract (NLCUE) @ 2% with (7.0 flowers/bee/min) while ASCUE @ 2% showed foraging rate slightly more *i.e.* (8.67 flowers/bee/min). On the other hand, in untreated mustard crop, the foraging rate of honeybees was recorded (10.00 flowers/bee/min) which was less than the foraging rate recorded in the mustard crop sprayed with various types of animal urine. Thus, it is clearly evident that the mustard crop sprayed with different types of animal urine were showed foraging rate of honeybees above 13.00 flowers/bee/min, while the cow urine based plant extracts *i.e.* NLCUE and APSCUE @ 2% showed less foraging rate of honeybees than foraging rate observed in untreated mustard crop *i.e.* 10.00 flowers/bee/min.

The foraging rate was increased drastically between 12:00-1:00PM, recording maximum foraging rate of (24.0 flowers/bee/min) in case of desi cow urine @ 20%, followed by (21.0 flowers/bee/min) in crops sprayed with holstein cow urine @ 20%, while the

crops sprayed with buffalo urine and jersey cow urine @ 20% recorded (19.0 and 19.67 flower/bee/min). Among animal urine, the lowest foraging rate was observed in crops sprayed with goat urine @ 20% (17.67 flower/bee/min), while in case of NLCUE and ASCUE @ 2% the foraging rate was recorded (9.0 and 10.67 flowers/bee/min) in comparison to the foraging rate observed in untreated crop (15.0 flower visit/min), while the cow urine based plant extracts of neem leaf and (*Trachyspermum ammi*) ajwain seed @ 1% recorded the foraging rate slightly below control (14.67 flowers/bee/min) and (13.0 flowers/bee/min), respectively..

Table 1: Effect of eco-friendly formulations on foraging rate of honeybee, *Apis mellifera* on mustard crop at Pantnagar, Uttarakhand

Treatments	Conc. (%)	Foraging rate Foraging rate (average no. of flower visited /bee/min) at different time intervals		
		9:00-10:00AM	12:00-1:00PM	03:00-04:00PM
Desi cow urine	20	13.00	24.00	12.67
Holstein cow urine	20	18.66	21.00	11.00
Jersey cow urine	20	15.00	19.67	13.00
Buffalo urine	20	13.00	19.00	12.00
Goat urine	20	13.00	17.67	10.66
NLCUE	01	11.33	13.00	12.00
ASCUE	01	13.33	14.67	11.67
NLCUE	02	07.00	09.00	08.00
ASCUE	02	08.67	10.67	06.67
Untreated	-	10.00	15.00	07.00
Sem ±		0.007	0.005	0.006
Cd at 5%		0.020	0.014	0.017
Cv		0.097	0.049	0.094

NLCUE-Neem Leaf Cow Urine Extract ASCUE- Ajwain Seed Cow Urine Extract

At time span of 3:00-4:00 PM, the maximum foraging rate was observed in the crop sprayed with jersey cow urine @ 20% (13.0 flowers/bee/min) followed by desi cow urine @ 20% (12.67 flower/bee/min), buffalo urine @ 20% (12.0 flowers/bee/min) and holstein cow urine (11.0 flowers/bee/min) whereas among the animal urine sprayed on mustard crop, the lowest foraging rate was recorded in goat urine @ 20% with (10.66 flowers/bee/min.). Foraging rate recorded on crops sprayed with cow urine based plant extracts *i.e.* NLCUE and APSCUE @ 1% was recorded (12.0 flowers/bee/min) and (11.67 flowers/bee/min.), respectively, while the lowest foraging rate (6.67 flowers/bee/min) and (8.0 flowers/bee/min) was recorded in the higher concentrations @2.0% of NLCUE and ASCUE. All eco-friendly formulations showed the highest foraging rate of honeybees in comparison to the foraging rate of honeybees was recorded on the untreated mustard crop (7.0 flowers/bee/min).

As far as foraging speed is concerned, maximum foraging speed was observed in mustard crop sprayed with holstein cow urine @ 20% with (5.0 sec/bee/flower) both at 12:00-1:00 PM and 3:00-4:00 PM. The lowest foraging speed was observed in crop sprayed with goat urine @ 20% with (2.0 sec/bee//flower). On analysis of foraging speed at different time span we found that at 9:00-10:00AM, the foraging speed of honeybee on crop sprayed with animal urine based formulations generally ranged from the minimum in goat urine @20% (2.0 sec/bee/flower) to the maximum (4.33 sec/bee/flower) in jersey cow urine @ 20%, while the foraging speed on crop sprayed with desi cow urine @ 20% was recorded (4.0 sec/bee/flower) followed by holstein cow urine (3.67 sec/bee/flower) and buffalo urine (2.33 sec/bee/flower). Among the cow urine based plant extracts *i.e.* NLCUE and ASCUE @ 1% and 2% sprayed on mustard crop, the maximum foraging speed was recorded (4.67 sec/bee/flower) in NLCUE @ 1%, followed by ASCUE@ 1% (03.33 sec/bee/flower), while NLCUE and ASCUE @ 2.0% showed the minimum foraging speed (2.67 and 2.33 sec/bee/flower). In the untreated crop the foraging speed of forager bees was recorded as (2.33 sec/bee/flower).

The foraging speed of honeybees between 12:00-1:00 PM was slightly increased with maximum in mustard crop treated with holstein cow urine @ 20% (5.0 sec/bee/flower), with comparable foraging speed of (4.67 sec/bee/flower) in desi cow urine @ 20% and NLCUE @ 1%. The minimum foraging speed was observed in crop sprayed with goat urine and jersey cow urine @20%

as (3.33sec/bee/flower) each followed by buffalo urine (3.67sec/bee/flower), while foraging speed of honeybees was recorded (4.33 sec/bee/flower) in ASCUE @1.0% followed by NLCUE @2.0% (4.00sec/bee/flower) and ASCUE @2% (3.67 sec/bee/flower) in comparison to foraging speed of honeybees calculated in untreated mustard crop (3.33 sec/bee/flower).

Table 2: Effect of eco-friendly formulations on foraging speed of honeybee, *Apis mellifera* on mustard crop at Pantnagar, Uttarakhand

Treatments	Conc. (%)	Foraging speed of bees (sec/bee/flower) at different time intervals		
		9:00-10:00AM	12:00-1:00PM	03:00-04:00PM
Desi cow urine	20	04.00	04.67	04.67
Holstein cow urine	20	03.67	05.00	05.00
Jersey cow urine	20	04.33	03.33	04.33
Buffalo urine	20	02.33	03.67	04.67
Goat urine	20	02.00	03.33	03.33
NLCUE	01	04.67	04.67	04.67
ASCUE	01	03.33	04.33	04.33
NLCUE	02	02.67	04.00	03.67
ASCUE	02	02.33	03.67	03.00
Untreated		02.33	03.33	03.67
Sem ±		0.005	0.006	0.006
Cd at 5%		0.016	0.019	0.018
Cv		0.296	0.276	0.247

NLCUE- Neem Leaf Cow Urine Extract ASCUE- Ajwain Seed Cow Urine Extract

After spraying of eco-friendly formulations on mustard crop, the foraging speed of honeybees was recorded between 3:00-4:00 PM with maximum foraging speed reported (5.0 sec/bee/flower) on crop sprayed with holstein cow urine @ 20%, while foraging speed of (4.67 sec/bee/flower) in crop sprayed with desi cow urine, buffalo urine @ 20% and NLCUE @ 1%, while the foraging speed of honeybees reported on crop sprayed with jersey cow urine @ 20 % and ASCUE @1% was observed (4.33 sec/bee/flower) followed by NLCUE @ 2% (3.67 sec/bee/flower), goat urine @ 20% (3.33 sec/bee/flower) and the minimum foraging speed of honeybees was recorded in ASCUE @2% (3.00 sec/bee/flower) in comparison to foraging speed calculated in untreated mustard crop (3.67 sec/bee/flower).

Discussion

The eco-friendly nature of two (*Azadirachtin indica*) neem based formulations NeemAzal™ T/S (1% azadirachtin) and NeemAzal™ granules (1% azadirachtin) was evaluated on honeybees, *Apis mellifera* L., under semi-field conditions by Abd-Allah *et al*, 2005 and found that these two formulation were completely safe and does not have any adverse effect on foraging behaviour and brood development of honeybees. Similarly, Singh *et al*, 2011 reported the effect of (*Azadirachtin indica*) neem product, ahook, bioneem and nimbidine on honeybees foraging in mustard crop and found them very safe for honeybees and recommended to farmers for eco-friendly management of mustard aphid, *Lipaphis erysimi* in mustard crop, while on the other hand the chemical possess a serious threat to honeybees and other pollinators. Thompson, 2003 reported that the exposure of bees to toxic chemicals resulted in wide range of impact on bees from inability of odour discrimination to disruption of their foraging and homing behaviour. Muranjan *et al*, 2006 were reported the slight toxicity of synthetic chemical, Chlorpyrifos to honeybees, *A. Cerana indica*. As far as, eco-friendly formulations such as animal urine collected from cow, buffalo and goat along with desi cow urine based neem (*Azadirachtin indica*) leaves and (*Trachyspermum ammi*) ajwain seed extracts were concerned, these formulations were used for the first time on mustard crop to study about their effect on foraging behaviour of honeybees but all of the tested eco-friendly formulations were found safe with no any adverse effect on honeybee foraging behaviour and they also showed a synergistic action to attract the forager honeybees towards the treated mustard crop in comparison to less visits of forager honeybees observed in untreated mustard crop.

Conclusion

The present field studies clearly revealed that among the tested eco-friendly formulations such as animal urine collected from desi, holstein and jersey breed cows and sprayed @20% and desi cow urine based (*Azadirachtin indica*) neem leaves and (*Trachyspermum ammi*) ajwain seed extracts @1% were found safe and had no any adverse effects on foraging behaviour of honeybees as they acted as an attractants and also promoted the foraging rate and speed of honeybees in mustard crop in comparison to foraging rate and speed of honeybees observed in untreated mustard crop. Thus, it may be concluded that these animal and plant based eco-friendly formulations can easily be used to increase the pollination efficiency of honeybees in this important oilseed crop of Uttarakhand.

The study further suggest that there is no adverse effect of eco-friendly formulations on the foraging behaviour *i.e* (foraging rate and speed) of honey bees, in treated mustard crop in turn it does seem to have a congenial effect on the forager honeybees as the rate and speed of forager on mustard crop sprayed with eco-friendly formulations increased,

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