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Quantitative evaluation of nectar secretion and production of nectar energy from Indian mustard flowers (*Brassica juncea* L.): A study in bioenergetics

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Abstract
Nectar secretion rhythms and nectar energetics of Indian mustard flowers, variety RH 0749 (recently developed and recommended by CCS HAU, Hisar) were studied during 2015-2016 Rabi Season at Research Area of Oilseeds Section, Department of Genetics and Plant Breeding, and Apiculture Laboratory of the Department of Entomology, CCS Haryana Agricultural University, Hisar, Haryana. The mean dry nectar-sugar (DNS) of 0.055 mg was produced per flower of *B. juncea* cv. RH-0749. Just opened flowers produced minimum amount of DNS (0.019 mg), while, it was maximum in the flowers after 4 hours of opening (0.099 mg). The energy reward per flower of *B. juncea* cv. RH-0749 varied from a minimum of 0.323 joules in just opened flower to a maximum of 1.661 joules after 4 hours of opening of flower. On an average, 0.913 joules energy was produced per *B. juncea* flower.

Keywords: *B. juncea*, Dry nectar sugar, Indian mustard, nectar, nectar energetics

1. Introduction
Indian mustard, *Brassica juncea* (L.) Czernj. & Cosson, popularly known as *rai*, *raya* or *laha* (Family Brassicaceae) is the oldest cultivated amphidiploid and is believed to have originated from *B. rapa* and *B. nigra* in Asia minor and southern Iran. Indian mustard (*B. juncea*) is a naturally autogamous species, yet in this crop frequent out-crossing occurs which varies from 5 to 30 per cent depending upon the environmental conditions and random variation of pollinating insects (Kumar *et al*., 2013) [1]. The bowl shaped flower of mustard is a suitable place for the landing of insect pollinators, especially honey bees (Roubik, 1989) [2]. Nectar is recognized as the main attractant for pollinators. The amount of nectar secreted and its sugar values are variable in different plant species and nectar secretion is also influenced by sex, age of flowers, climatic conditions and geographical locations (Eisikowitch and Masad, 1980 [3], and Sihag and Kapil, 1983 [4]).
Dhaliwal and Atwal (1972) [5] observed that the nectar sugar concentration in 'raya' (*B. juncea*) and 'toria' (*B. campestris* var. toria) was 53.10 and 56.40 per cent, respectively. Mukhametzyanova and Khamidov (1977) [6] reported that *B. napus* flowers secrete 0.23-0.60 mg nectar with an average sugar content of 49 per cent. Smaragdova (1977) [7] observed 0.362 and 0.103 mg nectar-sugar per flower in *B. campestris* var. dichotoma and *B. juncea*, respectively. Sihag and Kapil (1983) [4] observed 0.029-0.4 mg per flower in different *Brassica* species. Srawan and Sohi (1985) [8] reported that the total soluble solids in the nectars were 35.00, 43.00 and 55.00 per cent in toria, mustard and raya, respectively. Abrol (1986) [9] observed that nectar secretion was at its peak at 1000-1100 h, declined at mid-day, probably because of evaporation, and again reached at its peak at 1500 h in *B. juncea*. Khatkar (1996) [10] reported that the average amount of sugars in B.S.H-1, YSPB-1, toria, RH-8812, RH-819 and RH-30 were 0.0837, 0.0780, 0.0371, 0.0246, 0.0245 and 0.0235 mg per flower, respectively. He also observed that nectar secretion was lowest at 0900-1000 h and reached its peak at 1300 h.
Masierowska (2003) [11] studied the dynamics and abundance of nectar secretion as well as sugar productivity in flowers of brown mustard (*B. juncea*) cv. Małopolska and white mustard (*Sinapis alba*) cv. Borowska. In both cultivars, lateral nectarines produced more nectar than the median ones. Nectar secretion started at loose bud and peaked during anther dehiscence. Average amount of nectar secreted by 100 flowers of cv. Małopolska and cv. Borowska were 119.9 mg and 134 mg, respectively. One hundred flowers of cv. Małopolska and cv. Borowska secreted 28.4 mg and 24.9 mg of sugars in nectar, respectively. Abrol (2007) [12] examined twenty-four cultivars of *B. campestris* var. toria for their nectar secretion characteristics,

nectar sugar concentration, amount of sugar and energy per flower per day in relation to their attractiveness to honey bees, *A. mellifera* and *A. cerana indica.* The volume of nectar produced ranged from a minimum of 0.052 µl to a maximum of 0.120 µl per flower per day. Nectar sugar concentration ranged between 36.0-43.8 per cent and the amount of sugar ranged between 0.0198-0.0504 mg per flower per day. The energy reward varied from a minimum of 0.330 joules to a maximum of 0.845 joules per flower per day. He also suggested that cultivars with higher caloric rewards had a competitive edge over others in attracting foraging populations and consequently in pollination.

2. Materials and Methods

The present investigation was carried out with Indian mustard variety RH 0749 (recently developed and recommended by CCS HAU, Hisar) during 2015-2016 Rabi Season at Research Area of Oilseeds Section, Department of Genetics and Plant Breeding, and Apiculture Laboratory of the Department of Entomology, CCS Haryana Agricultural University, Hisar, Haryana (29°10'N, 75°46'E, 215.2 AMSL). The crop was sown in 1st week of October, 2015 with five replications. Crop was raised as per the practices recommended in Package of Practices of CCS HAU, Hisar.

The nectar secretion rhythms and nectar energetics of Indian mustard (RH 0749) flowers were estimated by adopting standard procedure as described below:

2.1 Nectar secretion rhythm of *B. juncea* cv. RH 0749 flowers

Quantitative estimation of dry nectar sugars (DNS) was done in flowers of RH 0749 by using the method of Roberts (1979) [13].

The flower buds which tend to open next day were tagged with the thread for identification and were covered with the butter paper bags in order to protect their nectar from honey bees and other insects. The flowers were collected from already marked plants in each replication consisting of 10 flowers at different day times *viz*., 0900, 1300 and 1700 h for two consecutive days because *Brassica* flowers remain open for 2 days only (Gill, 1991) [14]. These flowers were put in plastic tubes (5×2.5 cm size) and rinsed in 5 ml of distilled water. Each tube was tightly capped, shaked vigorously to rinse the nectaries and allowed to as such for 45 minutes. Nectar washed flowers were taken out from the tube and the rinsate was stored in the refrigerator to prevent the growth of micro organisms, until used for analysis. Total three replications and a blank was maintained for analysis of dry nectar sugar. The required amount of rinsate (1 ml) was transferred to test tube and 1 ml of 5 per cent phenol solution was added followed by 5 ml of concentrated sulphuric acid.

The final solution was mixed with the help of a test tube shaker. After digestion for 45 minutes to allow colour development, absorbance of the solution was measured with the help of Spectrophotometer 2203 (Jenway) at 490 nm against blank. The amount of sugar corresponding to observed absorbance was then estimated from the standard glucose curve to obtain the total amount of sugar per flower (expressed as milligram of glucose equivalent).

2.2 Standard curve

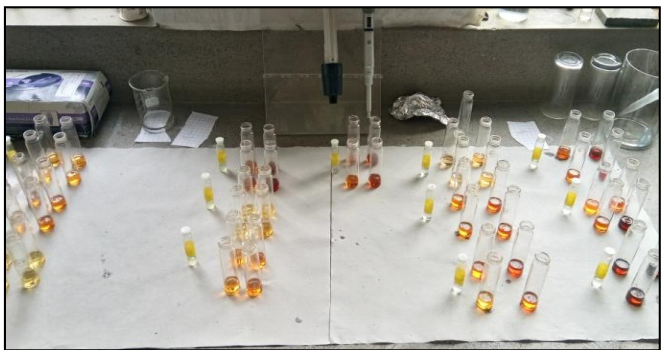
For preparing standard curve, 10 mg glucose (GR, Merck) was dissolved in 100 ml of distilled water. From this stock solution, aliquotes of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml were taken in separate test tubes. Final volume of each test tube was adjusted to 1 ml by adding required amount of distilled water. To the blank test tube, only 1 ml of distilled water was added instead of stock solution. Glucose values were estimated as already discussed above. Glucose values of nectar sugars in unknown samples were thus, determined by using this standard curve. Sugar values were represented as milligrams of glucose equivalent. The data was subjected to analysis of variance.

2.3 Energy production rhythms in *B. juncea* cv. RH 0749 flowers

The amount of energy produced by a *B. juncea* flower through its nectar was calculated by assuming that 1 mg of sugar (irrespective of the type) yields 4 cal or 16.74 joules of energy following the method of Heinrich (1975) [15]:

Energy/flower = amount of sugar×16.74 (joules)

RH 0749 flowers covered with butter paper bags



Final solution in test tubes

Absorbance measured at spectrophotometer

Plate 1: Determination of dry nectar sugar in flowers of *Brassica juncea*

3. Results and Discussions

3.1 Nectar secretion rhythms and nectar energetics of Indian mustard flowers

3.1.1 Nectar secretion rhythms

Nectar secretion was observed for two days in *B. juncea* flowers cv. RH-0749 because *Brassica* flowers remain open for 2 days only (Gill, 1991) [14] and the data presented in Table 1 and Figure 1 revealed that the average amount of dry nectar-sugar per flower of *B. juncea* cv. RH-0749 was significantly highest (0.099 mg) after 4 hours of opening, followed by 0.074 mg after 8 hours of opening, 0.058 mg after 24 hours of opening, 0.050 mg after 28 hours of opening, and 0.026 mg after 32 hours of opening and it was minimum (0.019 mg) at the time of flower opening. On an average, 0.055 mg of dry nectar-sugar was secreted by a *B. juncea* flower.

The present findings are in corroboration with the observations made by Tanda (1984) [16], Gill (1991) [14] and Khatkar (1996) [10] who found that nectar sugar was lowest at 0900-1000 h and reached a peak at 1200-1400 h of the day. Corbet (1978) [17] also observed changes in amount and composition of nectar from hour to hour and from day to day. Similar results were documented by Kubisova *et al.* (1980) [18] that the *B. napus* flowers secrete nectar for two days. In contrary to the present findings, Murrell and Nash (1981) [19] and Abrol (1986) [9] observed peak nectar-sugar at about 0900 h and 1000-1100 h on *B. campestris* and *B. juncea*, respectively. Radchenko (1964) [20] also observed nectar-sugar secretion was highest in freshly opened flowers and lowest at the time of closing.

Table 1: Dry nectar sugar and energy produced by the flowers of *Brassica juncea* variety RH-0749 during the year 2015-2016 at Hisar

***Brassica juncea* cv. RH-0749**		
Flower age	**Dry nectar sugar (mg/flower)**	**Energy (joules/flower)**
Just opened	0.019	0.323
04 h	0.099	1.661
08 h	0.074	1.224
24 h	0.058	0.972
28 h	0.050	0.846
32 h	0.026	0.435
Mean	0.055	0.913
SE(m)	0.001	0.019
CD (p= 0.05)	0.004	0.060

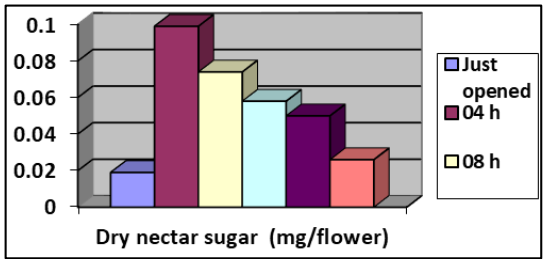


Fig 1: Dry nectar sugar in flowers of *Brassica juncea*

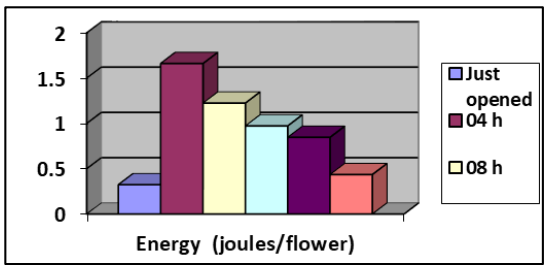


Fig 2: Nectar energetics in flowers of *Brassica juncea*

3.1.2 Nectar energetics

The energy produced by the flowers of *B. juncea* cv. RH-0749 was calculated by using the dry nectar-sugar per flower and the data is presented in Table 1 and Figure 2. The energy reward per flower varied from a minimum of 0.323 joules in just opened flower to a maximum of 1.661 joules after 4 hours of opening of flower. On an average, 0.913 joules energy was produced by a *B. juncea* flower. The present studies are in corroboration with the findings of Abrol (2007) [12] who reported that the energy reward varied from a minimum of 0.330 joules to a maximum of 0.845 joules per flower per day in *B. campestris* var. toria.

4. Conclusion

From the findings of research conducted, it was observed that the average amount of dry nectar-sugar per flower of *B. juncea* was significantly the highest (0.243 mg) after 4 hours of opening, followed by 0.203 mg after 8 hours of opening, and it was minimum (0.096 mg) at the time of flower opening. On an average, 0.055 mg of dry nectar-sugar was secreted by a *B. juncea* flower. The energy reward per flower varied from a minimum of 0.323 joules in just opened flower to a maximum of 1.661 joules after 4 hours of opening of flower. On an average, 0.913 joules energy was produced by a *B. juncea* flower.

5. References

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