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Determination of feeding performance of mustard aphid, *Lipaphis erysimi* (Kalt.) on different mustard varieties at water stress condition

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ABSTRACT

The more feeding of plant sap by aphid, the more secretion of honeydew. Determination of the amount of honeydew secreted by aphids in a specific crop variety is one of the best ways to assess the susceptibility of aphid infestation to the variety. An experiment was conducted in glass house and controlled growth room of Bangladesh Institute of Nuclear Agriculture (BINA) at 25 °C, RH 65–70% and 12:12 hrs daylight on 15 November 2016. Six mustard varieties were evaluated to determine the intensity of honeydew excretion by aphids for assessing susceptibility of aphids in mustard varieties under different water stress condition likely 100% field capacity (control), 50% field capacity (moderate drought) and 30% field capacity (severe drought). Aphid feeding and honeydew secretion were remarkably negatively affected by water stress. Weight of honeydew secreted by a mustard aphid during 24 hrs was reduced by 13% and 25% when subjected to moderate and extreme drought respectively compared to that at non-stressed control. The weight of honeydew secreted by a mustard aphid in 24 hrs in the varieties of *Brassica campestris*, *B. napus* and *B. juncea* was measured ranging 7.65 to 8.85 mg; 4.81 to 5.15 mg and 8.87 to 9.17 mg, respectively. It may be concluded that feeding performance of aphid and honeydew secretion were more in susceptible varieties and negatively affected by water stress. The varieties of *B. napus* are more resistant to aphid infestation.

Keywords: Aphid infestation, honeydew, mustard, drought stress

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1 Introduction

Mustard is a major oilseed crop and worldwide increasing trend in mustard production leading to 67.6 million tonne (Mt) in 2013/14 (Swati and Das, 2015). It has gained wider acceptance among farmers for its adaptability to irrigated and rain-fed areas as well (Prakash, 1980). This crop plays an important role in the agro-economy of Bangladesh and also in human health. Mustard aphid, *Lipaphis erysimi* (Kalt) is one of the destructive insect pests and is distributed worldwide (Singh and Sachan, 1994) including temperate as well as subtropical regions (Blackman and Eastop, 1984). Aphids are largely phloem feeders. Infestation of aphids in mustard crop is an important qualitative and quantitative yield-limiting factor (Singh, 2010).

Among the environmental constraints, drought effect on crop growth and production is worldwide (Ashraf and Harris, 2004; Farooq et al., 2009). Drought is a serious problem and challenge for successful agricultural production even in Bangladesh. It is hypothetically said that plant changes its cell or phloem composition to compensate against water stress which could affect aphid's reproduction (Vickers, 2011) and setting successful colony. So, in the current climate change scenario, the determination of relationship between aphid and drought is an important aim to the scientists for sustainable agriculture. Multiple interactions among herbivores, drought condition and their host plants in an agro-ecosystem could be linked through plant-based food chains which lead the researchers to conclude that host plant resistance should be a valuable tool in the management of this insect pest.

In this study, glass house experiments were designed to examine the interactive effects of mustard varieties and different water stress condition on honeydew secretion by aphids. The objectives were: 1) to evaluate the response of honeydew secreted by aphids to water stress condition and 2) to assess the susceptibility of aphid infestation to mustard varieties by excretion of honeydew.

2 Materials and Methods

An experiment was conducted following Complete Randomized Design (CRD) with 5 replications in glass house and controlled growth room of Bangladesh Institute of Nuclear Agriculture (BINA) on 15 November 2016. Six mustard varieties *viz*. Tori 7 and Porsha local of *B. campestris*; BINA Sharisha 4 and BARI Sharisha 13 of *B. napus*; BARI Sharisha 11 and BARI Sharisha 16 of *B. juncea* were evaluated to determine the intensity of honeydew secretion by aphids for assessing susceptibility of mustard varieties to aphid infestation under different water stress condition likely 100% field capacity (control), 50% field capacity (moderate drought) and 30% field capacity (severe drought).

2.1 Plant culture under water stress

After surface sterilization with Provax-200 WP (Thiram + Carboxin) @ 2g per 1kg seeds, seeds of each six varieties were sown separately in plastic pots (10 cm diameter × 12 cm height) filled with fertilized soil. The soil used as experimental material was sun dried, grounded, sieved, and well mixed at the beginning of the experiment. The characteristics of the growth medium and basal nutrients (using urea, di-ammonium phosphate, and potassium sulfate as sources of N, P, and K, respectively) applied in solution to each pot were according to Shabbir et al. (2015). Seedlings were thinned to three per pot at the cotyledon stage and all pots were irrigated twice per week with water as needed. After two weeks, plants were assigned separate irrigation regimes and three water level conditions mentioned above were maintained in the soil up to flowering stage. Initial water (100% field capacity) was measured by oven dry method and other 50% and 30% field capacity of soil were maintained by calibration. All the plants were cultured without any infestation of aphids.

2.2 Preparation of feeding chamber

The feeding chamber for each of six varieties grown under three different water regimes was prepared separately inside a controlled growth room maintaining 25 °C, RH 65–70% and 12:12 hrs daylight to collect honeydew. A separate inverted cup (upper and lower diameters of 6 and 4 cm, respectively, and a height of 6 cm) for each of six varieties and each three water stress regimes was taken. The inverted cup was perforated at the top through which a twig with aphids was set. At the base of the inverted cup, a previously weighted Para film was unfolded on which secreted honeydew would drop.

2.3 Placing twigs into feeding chambers

At each treatment, twenty (20) nymphs of aphid previously starved for 2 hrs were released into the excised twig of mustard plant. One hour after setting of nymphs, the twigs were suspended into the feeding chambers through a hole at the top of the inverted cup. Cotton bud was placed in the hole to prevent escaping of insects.

2.4 Preparation of transpiration chamber

The transpiration (control) chamber for each of six varieties grown at three different water regimes with five replications were also prepared separately to collect transpiration water. An insect free twig for each of six varieties grown at three different water regimes was placed horizontally into the cup wrapped with previously weighted Para film separately.

2.5 Measurement of secreted honeydew

The aphids were allowed to feed and secrete honeydew droplets on stretched Para film. After 24 hrs, the weights of stretched Para film with honeydew droplets were measured. Again, at the same time, the Para film with transpiration water collected from the corresponding cup was weighted. Average weight of secreted honeydew per insect was measured using the following formula (Begum and Wilkins, 1998; Cao, 2013) expressed in milligrams.

$$\text{Wt. of honeydew} = (\text{A} - \text{B}) - (\text{X} - \text{Y})$$

Plate 1. Maintenance of water stress treatments by continuous weighing and watering

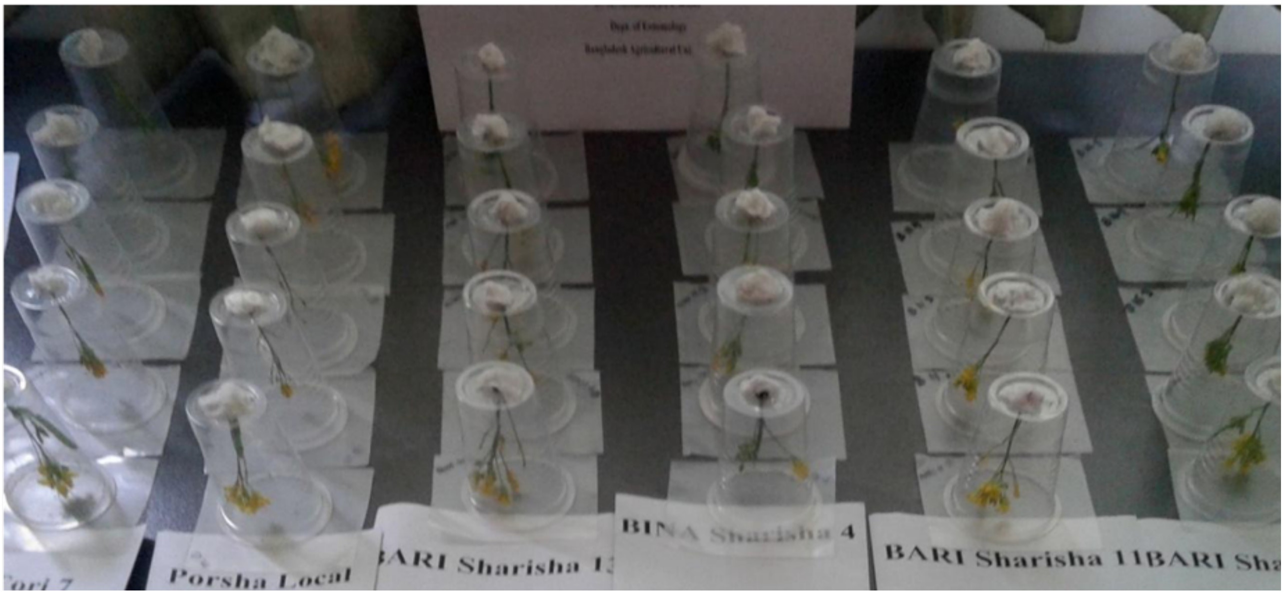

Plate 2. Feeding chamber of aphids with different mustard varieties at water stress condition

Plate 3. Artificial aphid infestation to the mustard inflorescence (left), Placement of feeding chamber on Para film paper (middle), and Honeydew secretion by aphid on Para film paper (right)

where, A = Wt. of Para film with secreted honeydew, B = Wt. of Para film before secretion, X = Wt. of Para film with collected transpiration water, and Y = Wt. of Para film without transpiration water.

2.6 Statistical analyses

The glass house experiments were oriented with Completely Randomized Design (CRD). All the data obtained from the experiments were checked first and then were transformed whenever necessary. The data were analyzed using 'Statistix 10' software on a computer. Interaction effects of drought and mustard varieties on weight of honeydew secretion (in laboratory condition) were analyzed using two-way ANOVA. Differences between treatments were compared using Duncan's multiple range test (DMRT) and least significant difference (LSD) test at the 5% level. The graphs were prepared using 'SigmaPlot' software.

3 Results

3.1 Response of honeydew secretion to water stress

The effect of water stress on honeydew secretion of mustard aphids was significant ($P<0.001$). Drought stress caused decreases in Honeydew and the results are shown in Table 1. Weight of honeydew secreted by a mustard aphid in 24 hrs was reduced by 13% and 25% when subjected to moderate (50% FC) and extreme (30% FC) drought respectively compared to that at non-stressed control (100% FC).

Interaction effects of the drought stress and all mustard varieties tested in the present studies on honeydew secretion of mustard aphids was significant ($P<0.001$) (Fig. 1). The weight of honeydew secreted by a mustard aphid in 24 hrs declined remarkably in all the varieties of *Brassica campestris*, *B. napus* and *B. juncea* under drought stress like moderately water stress (50% FC) and severe water stress (30% FC) as compared to non-stressed control plants (100% FC). This trend of reduction significantly was more in severe water stress from moderately stress. The weight of honeydew in all the tested varieties like Tori 7, Porsha Local, BINA Sharisha 4, BARI Sharisha 13, BARI Sharisha 11 and BARI Sharisha 16 was reduced by 23, 12, 11, 17, 8 and 8%, respectively under moderate drought stress; while it was reduced by 30, 24, 21, 24, 23 and 24%, respectively under severe drought stress as compared to control condition (100% FC).

3.2 Susceptibility to mustard varieties

There were significant difference ($P<0.001$) regarding honeydew excretion by mustard aphid among all the mustard varieties tested (Table 2). The weight of honeydew secreted by a mustard aphid in 24 hrs in the mustard species like *B. campestris*, *B.napus* and *B. juncea* was measured ranging 7.65 to 8.85 mg; 4.81 to 5.15 mg and 8.87 to 9.17 mg, respectively. The varieties of *B. campestris* and *B. juncea* responded maximum to honeydew excretion; whereas those of *B. napus* displayed minimum. Among the varieties, BARI Sharisha 11 displayed maximum (9.17 mg) honeydew excretion followed by that of BARI Sharisha 16 (8.87 mg) and Porsha Local (8.85 mg); while, the minimum weight was measured from that of BINA Sharisha 4 (4.81 mg).

The Fig. 1 showd the interaction effects of the drought stress and all mustard varieties tested on the weights of honeydew produced by mustard aphid. The mustard aphid displayed different significant ($P<0.001$) weight of honeydew among the host varieties due to different water stress conditions. Among the tested varieties, the maximum weight of honeydew secreted by a mustard aphid in 24 hrs was recorded in the variety, BARI Sharisha 11 (10.24 mg) which was statistically similar to that of Porsha local (10.08 mg) and lowest was in BINA Sharisha 4 (5.38 mg) followed by BARI Sharisha 13 (5.98 mg) when grown in control condition. Again, a mustard aphid secreted maximum weight of honeydew during 24 hrs in the variety, BARI Sharisha 11 (9.38 mg), which was statistically similar to that in BARI Sharisha 16 (9.12 mg) and minimum in BINA Sharisha 4 (4.80 mg) followed by BARI Sharisha 13 (4.94 mg), when imposed moderate drought stress (50% FC). Mustard aphid displayed maximum weight of honeydew during 24 hrs in the variety, BARI Sharisha 11 (7.90 mg) followed by that in Porsha Local (7.64 mg) and BARI Sharisha 16 (7.54 mg) and minimum in BINA Sharisha 4 (4.24 mg) followed by BARI Sharisha 13 (4.52 mg) when imposed to severe drought stress (30% FC).

Interaction between drought stress and mustard varieties resulted that mustard aphid fed minimum in varieties of *Brassica napus* as compared to those of *B. campestris*, and *B. juncea*. The varieties, BARI Sharisha 11, Porsha local and BARI Sharisha 16 were more vulnerable to aphid infestation. The rate of phloem feeding of mustard aphid declined with the intensity of water stress level.

4 Discussion

Among the many techniques developed to measure the feeding response of aphid on resistant and susceptible plants are those based on weight or volume determination of honeydew excreted (Paguia et al., 1980; Begum and Wilkins, 1998). The weight of honeydew secreted by aphid depends on host varieties, nutrient availability, aphid developmental stages, biotic and abiotic stress (Loxdale et al., 1998; Edwards, 2001; Wilson et al., 2003), the time spent by aphid in the ingestion of sieve element, sieve element content (Zhou et al., 2015), honeydew droplet frequency, vol-

Table 1. Effect of water stress on honeydew secretion by mustard aphid

Water stress level	Honeydew secreted (mg/24 hrs/aphid)	Honeydew secretion reduction over control (%)
100% FC (Control)	8.49 a	–
50% FC	7.37 b	-13
30% FC	6.40 c	-25
CV (%)	3.2	
Level of significance	***	
$LSD_{0.05}$	0.1225	
SE±	0.061	

Mean values sharing similar letters in a column are statistically non-significant ($P \leq 0.05$); *** = Significant at 1% level of significance; SE = Standard Error, LSD = Least Significant Difference, FC= Field capacity

Table 2. Varietal effect of *Brassica* sp. on honeydew secretion by mustard aphid

Mustard species	Varieties	Honeydew secreted (mg/24 hrs/aphid)
B. Campesties	Tori 7	7.65 c
	Porsha local	8.85 b
B. napus	BINA Sharisha 4	4.81 e
	BARI Sharisha 13	5.15 d
B. juncea	BARI Sharisha 11	9.17 a
	BARI Sharisha 16	8.87 b
CV(%)		3.2
Level of significance		***
$LSD_{0.05}$		0.1732
SE±		0.087

Mean values sharing similar letters in a column are statistically non-significant ($P \leq 0.05$);
*** = Significant at 1% level of significance; SE = Standard Error, LSD = Least Significant Difference

ume of droplets as well. Honeydew results showed that droplet frequency was significantly reduced in response to drought. This reduction in honeydew frequency was also accompanied by a reduction in droplet volume. These findings were supported by those of Vickers (2011) who studied on behavioural analysis of aphids and showed that aphid took less time of feeding when they fed on stressed host plants.

Our studies revealed that a mustard aphid secreted honeydew ranging 10.24 to 4.24 mg during 24 hrs when tested varieties interacted with drought stress. These findings are supported by Volk et al. (1999) who showed that the weight of honeydew was considerably different among the 4 aphid species *M. fuscoviride* (880 μg per aphid per hour), *B. cardui* (223 μg per aphid per hour), *A. fabae* (133 μg per aphid per hour), and *M. tanacetaria* (46 μg per aphid per hour). Besides this, in another study, Zhou et al. (2015) found that Mean weight of honeydew (mg/drop), excretion frequency per aphid (drops/24 hrs) and mean weight of honeydew (mg/24 hrs) were 1.35, 20.60 and 30.54 respectively.

Honeydew excretion is widely used as a measurement of feeding activity and consequently as an index for resistance and susceptibility of a crop variety to homopteran pests (Liu et al., 1994). In the present studies, significant differences were recorded in the quantity of honeydew excreted by individual aphid over 24 hrs feeding on mustard varieties. Similar observations were found by Begum and Wilkins (1998). Generally, when aphids feed on a preferred host plant, time from start of penetration to the phloem sap ingestion is shorter and the mean duration of phloem ingestion per insect is longer resulting higher honeydew weight; when compared with feeding on a non-preferred host plant (Seo et al., 2010; Ghaffar et al., 2011; Cao et al., 2013). So it is no doubt to say that non-preferred host variety for aphid is the resistant one.

The phenomenon where the insect like aphid receives maximum benefit from the crop variety *viz.* more phloem sap ingestion, more honeydew excretion is the sign of more susceptibility of the variety to insect (Cheng et al., 2001; Lü et al., 2011; Cao, 2013; Cao et al., 2013). It has been known for many years that interactions between plants and insects depend on two reciprocal responses: host plant selection by insects and the chemical defenses of their host plants

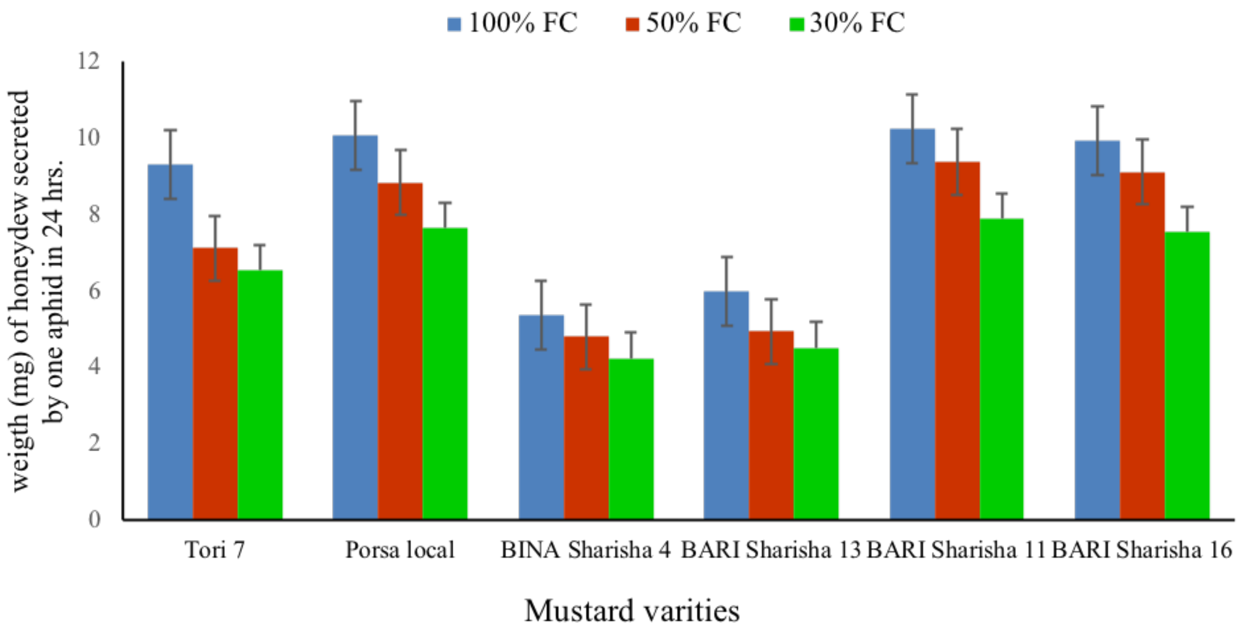

Figure 1. Effect of varieties and water stress on measurement of honeydew secretion by mustard aphid

to those insects. The previous studies have shown that facilitation of feeding behavior and performance is related to chemical changes in host plants (Zhao et al., 2000; Liu et al., 2002). Zhao et al. (2000) mentioned that the higher concentration of amino acids in host plant facilitates the insect to feed more phloem sap and excrete more honeydew. These observations suggest that changes in amino acid profiles in host plants are caused by feeding activities of phloem sap feeder. Almost similar results have been revealed by Zhou et al. (2015) who mentioned the sugar concentration might cause these changes.

5 Conclusion

Aphid feeding and honeydew secretion were remarkably affected by water stress. Weight of honeydew secreted by a mustard aphid during 24 hrs was reduced by 13% and 25% when subjected to moderate and extreme drought, respectively compared to that at non-stressed control. Further, a mustard aphid secreted honeydew ranging 7.65 to 8.85 mg; 4.81 to 5.15 mg and 8.87 to 9.17 mg in the varieties of Brassica campestries, *B. napus* and *B. juncea*, respectively during 24 hrs. It may be concluded that honeydew secretion were more in susceptible varieties and negatively affected by water stress. The varieties of *Brassica napus* were more resistant to aphid infestation.

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