Incidence and Host Preference of Red Pumpkin Beetle, *Aulacophora foveicollis* (Lucas) on Cucurbitaceous Vegetables

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Abstract: Three cucurbitaceous vegetables viz. sweet gourd (BARI Misti Kumra-1, BARI Misti Kumra-2 and Local Misti Kumra), bitter gourd (BARI Karola-1, Taj Karola-88 and Local Karola) and bottle gourd (BARI Lau-3, BARI Lau -4 and Local Lau) were selected to conduct a research on the incidence and host preference of red pumpkin beetle. In the field experiment at all the three stages, the highest incidence of red pumpkin beetle per plant was observed on sweet gourd and lowest on bitter gourd. The maximum number of beetle was observed on Local Misti Kumra at all the three stages and the lowest number of beetle was found on BARI Karola-1 at seedling stage and on Taj Karola-88 at flowering stage and on Local Karola at fruiting stage in the field. In net cage experiment, the highest and lowest number of beetle was also observed on sweet gourd and bitter gourd respectively. In the net cage among the nine varieties, the maximum number of beetle was observed on Local Misti Kumra. At all the three stages the highest percentage of leaf infestation was found on sweet gourd and the lowest on bitter gourd in the field. Among the nine varieties, the highest percentage of leaf infestation was recorded on Local Misti Kumra at all three stages in the field. The highest food consumption was recorded on sweet gourd among the crops and the highest leaf area damage was found on Local Misti Kumra in net cage experiment among the nine varieties. Among three cucurbit crops the total and daily food consumption was found highest on sweet gourd in the laboratory. The total and daily food consumption was the highest on Local Misti Kumra whereas the lowest was recorded on Local Karola among the nine varieties.

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1. Introduction

Red pumpkin beetle (RPB), *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae) is a common and major pest of a wide range of cucurbits, specially sweet gourd, bottle gourd, bitter gourd, white gourd, water melon and musk melon. It is polyphagous in nature (Doharey, 1983). Both larval and adult stages are injurious to the crop and cause severe damage to almost all cucurbits at seedlings, young and tender leaves and flowers. (Rahaman and Prodhan, 2007; Rahman et al. 2008). The adult beetles feed on the leaves making irregular holes and also attack the flowers and flower buds.

But the larvae feed on root tissue and cause direct damage to the newly developed seedlings (Narayanan and Batra, 1960). Damage sometimes becomes very severe if it is not controlled timely. Losses due to infestation are quite evident which may reach up to 35-75 % at seedling stage (Yamaguchi, 1983). At the advent of spring the beetles defoliate the cucurbit seedlings to such an extent that sometimes the crop has to be resown for 3 to 4 times (Parsad and Kumar, 2002; Mahmood *et al.*, 2005). It makes the growers late marketing of the vegetables as well as lessen their income. The pest, however, occurs throughout the year and causes severe damage to the crops especially at seedling stage (Rajak, 2001).

To manage this pest properly, it is necessary to have an adequate knowledge on its incidence and hostplant preference for feeding and oviposition behaviour. Some studies on the different factors relating to the abundance of red pumkin beetle in different crops have already been done (Khan, 2011; Mandal, *et al.* 2012; Rathod and Borad, 2010; Khan *et al.* 2012; Khursheed and Nisar 2013). But unfortunately, information on incidence and host preference of this pest is limited. Considering the above situation in present study an attempt was made to observe the incidence and host preference for feeding, ovipositon and food consumption of red pumpkin beetle on different varieties of cucurbits in the field as well as in the laboratory.

2. Materials and Methods *Development of the plants*

The research was carried out in the field and laboratory of the Department of Entomology, Bangladesh Agricultural University, Mymensingh. Three cucurbitaceous vegetables viz. sweet gourd, Cucurbita maxima (BARI Misti Kumra-1, BARI Misti Kumra-2 and Local Misti Kumra), bitter gourd, Momordica charantia (BARI Karola-1, Taj Karola-88 and Local Karola) and bottle gourd, Lagenaria siceraria (BARI Lau-3, BARI Lau -4 and Local Lau) were selected to conduct this research. Seeds were collected from Bangladesh Agriculture Research Institute, Gazipur and commercial seed store of Mymensingh town. The field experiments were laid out in a Randomized Complete Block Design (RCBD) with 3 replications in the field. The whole experimental plot was 26.75 m \times 9.05 m, which was divided into 3 equal blocks. Each block was divided into 9 plots. Thus, there were 27 (9×3) unit plots altogether in the experiment. The size of a unit plot was 2.35 m \times 2.35 m. Block to block and plot to plot distance 1 m and 0.7 m were maintained to facilitate different intercultural operations. Three pits were prepared per plot.

Incidence of the red pumpkin beetle In the open field

Nine varieties of three cucurbitaceous crops such as sweet gourd, bitter gourd and bottle gourd were used to carry out this field experiment. The number of the beetle per plant was counted at three different growth stages of host plant viz. at seedling stage, at flowering stage and at fruiting stage. Thus mean number of beetle plant⁻¹ was calculated on three cucurbits. Data were recorded from 15 days after transplanting. The experiment was conducted with three replications. Pooled mean data for three crops were calculated from the mean values of three varieties of each crop.

In net cage

Plants of nine varieties of three cucurbitaceous crops viz, sweet gourd, bitter gourd and bottle gourd were taken for net cage experiment. Plants of these varieties were developed in the pots. Thereafter, sixty red pumpkin beetles were released on the plants encaged with a mosquito net. This experiment was done with three replications. The number of the beetles found per plant of each variety was recorded. Data were recorded at 1, 2, 3, 4, 5, 6 and 7 days after release (DAR). Average number of beetle plant⁻¹ was also calculated. Pooled means for three crops were calculated from the mean values of three varieties of each crop.

Host preference of Red Pumpkin Beetle Percentages of leaf infestation at different stages of plants in open field

Nine varieties of three cucurbits were used to conduct this experiment. Percentages of infested leaf in the open field of the plants were determined by visual observation at seedling, flowering and fruiting stage. Data were collected daily basis at three stages up to seven days. Firstly total number of leaf and then the number of infested leaf was counted and finally mean percentages of infested leaf was calculated. Pooled means for three crops were calculated from the mean values of three varieties of each crop.

Leaf area (mm²) damage in net cage

All the varieties used in the field experiment were also used to conduct this net cage experiment. The total leaf area damaged plant⁻¹ by the beetle was measured with the help of square millimeter graph paper after 7 days of beetle release at the seedling stage. Finally average leaf area damage plant⁻¹ was also calculated. Pooled means for three crops were calculated from the mean values of three varieties of each crop.

Food consumption in the laboratory

To estimate the food consumption, two adult beetles (male & female) were released in each petridish containing a leaf each of nine varieties. Three petridishes were maintained for each variety. Fresh leaves were supplied daily. The cut end of leaf petiole was provided with water soaked cotton pad to prevent withering of leaf. Food consumption was determined measuring the leaf area eaten by adult beetle using square millimeter graph paper and it was expressed as mm^2 . The preferred variety was determined depending on the amount of leaf area eaten by the adult beetle. This experiment was carried out up to seven days in the laboratory. Pooled means for three crops were calculated from the mean values of three varieties of each crop.

Data analysis

Data obtained from different experiments were analyzed using a statistical package program MSTAT-C. The mean values were ranked by Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

Incidence of red pumpkin beetle in the open field *Among different crops*

Among all the three stages of the tested three crops, the highest number of beetle was found on sweet gourd and the lowest number was on bitter gourd (Table 1). At seedling stage the highest number (3.57 beetle plant⁻¹) of beetle was observed on sweet gourd and the lowest (1.05 beetle plant⁻¹) on bitter gourd. Similarly at flowering stage the maximum

number of beetle (3.35 beetle plant⁻¹) was found on sweet gourd and the lowest (1.03 beetle plant⁻¹) was on bitter gourd. The highest number of beetle (3.14 beetle plant⁻¹) was also observed on sweet gourd and the lowest (0.92 beetle plant⁻¹) on bitter gourd at fruiting stage. So, sweet gourd was the most preferred and bitter gourd was the least preferred host among the three crops. The result supported the previous similar work done by Hasan *et al.* 2012. Begum (2002) also found the similar result from her study that sweet gourd was the highly preferred host to red pumpkin beetle among the five tested cucurbits (viz., sweet gourd, ash gourd, sponge gourd, snake gourd and cucumber).

Among different varieties

The number of beetle plant⁻¹ found at different stages (seedling stage, flowering stage and fruiting stage) of plant on nine different cucurbit varieties varied significantly under field condition. The average number of beetle plant⁻¹ found at three different stages showed significant difference. At seedling stage among nine varieties, the highest number of beetle plant⁻¹ (4.0 beetle plant⁻¹) was observed on Local Misti Kumra followed by BARI Misti Kumra-2 (3.57 beetle plant⁻¹) and BARI Misti Kumra-1(3.14 beetle plant⁻¹) which varied significantly. The lowest number of beetle was found on BARI Karola-1(0.95 beetle plant ¹) followed by Taj Karola-88 (1.0 beetle plant⁻¹) and Local Karola $(1.19 \text{ beetle plant}^{-1})$ which were statistically similar. The maximum number of beetle (3.67 beetle plant⁻¹) was recorded on Local Misti Kumra followed by BARI Misti Kumra-2 (3.29 beetle plant⁻¹) and BARI Misti Kumra-1 (3.10 beetle plant⁻¹) at flowering stage. There was no significant variation between BARI Misti Kumra-2 and BARI Misti Kumra-1. The lowest number of beetle was found on Taj Karola-88 (0.81 beetle plant⁻¹) followed by BARI Karola-1(1.05 beetle plant⁻¹) and Local Karola (1.24 beetle plant⁻¹).

At fruiting stage, the highest number of adult beetle (3.33 beetle plant⁻¹) was observed on Local Misti Kumra followed by BARI Misti Kumra-2 (3.14 beetle plant⁻¹) and BARI Misti Kumra-1 (2.95 beetle plant⁻¹) which were statistically identical. The lowest number of beetle was found on BARI Karola-1 (0.81 beetle plant⁻¹) followed by Taj Karola-88 (0.91 beetle plant⁻¹) and Local Karola (1.05 beetle plant⁻¹). So, among the nine varieties Local Misti Kumra was highly preferred and the least preferred variety was BARI Karola-1 followed by Taj Karola-88 and Local Karola. Among the three stages irrespective of crops and varieties maximum incidence of beetle was observed at seedling stage and minimum at fruiting stage.

Incidence of red pumpkin beetle in net cage *Among different crops*

Mean number of beetle plant⁻¹ found among three cucurbit crops under confined condition in the net cage varied significantly. The highest number of beetle (2.91 beetle plant⁻¹) was observed on sweet gourd and the lowest number was on bitter gourd. So, sweet gourd was the most preferred host while bitter gourd was the least preferred by red pumpkin beetle among the three cucurbits. Saljogi and Shahbaz (2007) were also observed the lowest attack by red pumpkin beetle on bitter gourd in their study. Similar trend of results also obtained on host preference by Khan. et al. (2011). They reported that sweet gourd and musk melon were found to be the most preferred host of red pumpkin beetle and bitter gourd was found as nonpreferred. Khan (2012), in his another work in field also found sweet gourd in the most preferred group and bitter gourd in the non-preferred group by the red pumpkin beetle which agrees with the present study.

Among different varieties

The incidence of red pumpkin beetle on the plants of nine different varieties of cucurbit crops under confined condition in the net cage varied significantly (Table 4). In the net cage among nine varieties, the maximum number of beetle (3.38 beetle plant⁻¹) was observed on Local Misti Kumra followed by BARI Misti Kumra-1 (2.76 beetle plant⁻¹) and BARI Misti Kumra-2 (2.57 beetle plant⁻¹). The average number of beetle plant⁻¹ found on BARI Misti Kumra-1 and BARI Misti Kumra-2 were not significantly different. The minimum number of beetle was found on Local Karola $(0.57 \text{ beetle plant}^{-1})$ followed by Taj Karola-88 and BARI Karola-1(0.67 beetle plant⁻¹) which were statistically similar. Therefore, among nine the varieties Local Misti Kumra was the most suitable and Local Karola was the least suitable variety for red pumpkin beetle.

Percentage of leaf infestation in the field Among different crops

Percentage of leaf infestation caused by red pumpkin beetle under field condition varied significantly at three different stages of crop growth (Table 5). At seedling stage, the highest percentage of leaf infestation was observed (33.37%) on sweet gourd and the lowest on bitter gourd (5.85%). At flowering stage, the highest percentage of leaf infestation was found (14.07%) on sweet gourd and the lowest percentage of leaf infestation was observed on bitter gourd (4.92%). At fruiting stage, the highest percentage of leaf infestation was found (13.87%) on sweet gourd and lowest on bitter gourd (4.77%). The results indicated that sweet gourd was the most suitable host for red pumpkin beetle at all the three stages of cucurbit crops. This result supported the previous study by Khan et al. (2011).

Among different varieties

Percentage of leaf infestation on nine different cucurbit varieties varied significantly at three different stages of crop growth (Table 6). The highest percentage of leaf infestation at seedling stage was observed on Local Misti Kumra (36.86%) followed by BARI Misti Kumra-1 (34.76%) and the lowest was on BARI Karola-1(5.12%) followed by Local Karola (6.14%) and Taj Karola-88 (6.27%). At flowering stage, the highest percentage of leaf infestation was observed on Local Misti Kumra (15.56%) followed by BARI Misti Kumra-2 (13.89%) and BARI Misti Kumra-1(12.78%). The lowest percentage of leaf infestation was observed on Taj Karola-88 (4.05%) followed by BARI Karola-1(4.33%) and Local Karola (6.37%) which were statistically similar. At fruiting stage, the maximum percentage of leaf infestation was found on Local Misti Kumra (17.48%) followed by BARI Misti Kumra-2 (12.18%) and minimum percentage of leaf infestation was observed on BARI Karola-1 (3.99%) followed by Taj Karola-88 (4.11%) and Local Karola (6.19%). The highest percentage of leaf infestation was observed on Local Misti Kumra at all three stages and the most damaging stage was seedling stage.

Leaf area (mm²) damage in net cage Among different crops

The highest leaf area damage was found on sweet gourd (686.44 mm²) and the lowest was on bitter gourd (39.42 mm²). The Leaf area damage found on three crops varied significantly (Table 7). Host preference rank was; sweet gourd >bottle gourd> bitter gourd. This result agrees with the results of Khan *et al.* (2011). Aziz (2009) did the similar type of study on the host preference of *Aulacophora foveicollis* Lucas (Coleoptera, Galerucidae) on melon *Cucumis melo*, snake cucumber *C. flexuosus*, cucumber *C. sativus* and bottle gourd *Lagenaria siceraria* and recorded the preference ranking as C. m. flexuosus> C. melo > C. sativus > L. siceraria which also supported the results of the present study.

Among different varieties

Under confined condition in the net cage leaf area damage found on nine varieties differed significantly (Table 8). The highest leaf area damage was found on Local Misti Kumra (737.24 mm²) followed by BARI Misti Kumra-1 (698.14 mm²) and the lowest leaf area damage was on BARI Karola-1(31.67 mm²) followed by Taj Karola-88 (39.83 mm²) and Local Karola (46.75 mm²). This result indicated that Local Misti Kumra was the most preferred and BARI Karola-1 was the least preferred host for red pumpkin beetle among the nine varieties. The present findings are also in agreement with the results of Khan *et al.* (2011).

Food consumption in the laboratory On the leaves of different crops

The total food consumption was the highest on sweet gourd (294.55 mm²) and the lowest (33.19 mm²) was recorded on bitter gourd. The highest (42.08 mm²) daily food consumption was also observed on sweet gourd and the lowest (4.74 mm²) food consumption was on bitter gourd (Table 9). Total and daily food consumption by the red pumpkin beetle among three crops differed significantly. This result again indicated that sweet gourd was the most preferred while bitter gourd was the least preferred host for red pumpkin beetle.

On the leaves of different varieties

The total food consumption by red pumpkin beetle on nine different cucurbit varieties in laboratory experiment is shown in table 10. The total food consumption was highest on Local Misti Kumra (336.29 mm²) followed by BARI Misti Kumra-2 (283.35 mm^2) and BARI Misti Kumra-1 (264.00 mm^2) whereas the lowest total food consumption (29.07 mm²) was recorded on Local Karola followed by Tai Karola-88 (34.75 mm²) and BARI Karola-1 (35.75 mm²). The highest average daily food consumption was recorded on the variety Local Misti Kumra (48.04 mm²) followed by BARI Misti Kumra-2 (40.48 mm²) and BARI Misti Kumra-1 (37.71 mm²) whereas the lowest daily food consumption (4.15 mm²) recorded on Local Karola followed by Taj Karola-88 (4.96 mm²) and BARI Karola-1 (5.11 mm²). Result on food consumption indicated that RPB consumed the highest amount of total and daily food from Local Misti Kumra. So, Local Misti Kumra was the most preferred host for red pumpkin beetle.

From the results it is clear that RPB has preference for different varieties of cucurbits as their host for feeding and the most favorable host variety was Local Misti Kumra followed by BARI Misti Kumra-2 and BARI Misti Kumra-1 whereas, the least preferred hosts were BARI Karola-1 followed by Taj Karola-88. Remaining varieties were moderately preferable to red pumpkin beetle.

4. Conclusion

This finding provides important information on the pest red pumpkin beetle and its host cucurbits which will be useful in conducting future research on this particular pest as well as in the ecofriendly and sustainable management of the pest.

	Number of beetle plant ⁻¹		
Crop	Seedling stage	Flowering stage	Fruiting stage
Sweet gourd	3.57 a	3.35 a	3.14 a
Bitter gourd	1.05 c	1.03 c	0.92 c
Bottle gourd	2.29 b	2.18 b	1.86 b
Mean	2.30	2.19	1.97
SE	0.73	0.67	0.64
LSD value	0.33	0.33	0.48
Level of significance	0.01	0.01	0.01
CV (%)	5.94	6.29	10.23

Table 1. Average number of beetle plant⁻¹ found at different stages on three cucurbit crops in open field

Means followed by common letter(s) are not significantly different

Table 2. Average number of beetle plant⁻¹ found at different stages on nine cucurbit varieties in open field

		Number of beetle plant ⁻¹		
Crop	Variety	Seedling stage	Flowering stage	Fruiting stage
	BARI Misti Kumra-1	3.14 c	3.10 b	2.95 a
Sweet gourd	BARI Misti Kumra-2	3.57 b	3.29 b	3.14 a
	Local Misti Kumra	4.00 a	3.67 a	3.33 a
	BARI Karola-1	0.95 f	1.05 de	0.81 d
Bitter gourd	Taj Karola-88	1.00 f	0.81 e	0.91 d
_	Local Karola	1.19 f	1.24 d	1.05 d
	BARI Lau-3	2.05 f	2.05 c	1.62 c
Bottle gourd	BARI Lau-4	2.29 de	2.10 c	1.81 bc
	Local Lau	2.52 d	2.38 c	2.14 b
Mean		2.30	2.19	1.97
SE		0.37	0.34	0.33
LSD value		0.24	0.33	0.48
Level of significance		0.05	0.01	0.01
CV (%)		5.94	6.29	10.23

Means followed by common letter (s) are not significantly different.

Table 3. Mean number of beetle plant⁻¹ found on the leaf of three cucurbit crops in net cage

Crop	Number of beetle plant ⁻¹ in net cage
Sweet gourd	2.91 a
Bitter gourd	0.64 c
Bottle gourd	1.83 b
Mean	1.79
SE	0.66
LSD value	0.42
Level of significance	0.01
CV (%)	9.99

Means followed by common letter (s) are not significantly different

Table 4. Average beetle plant⁻¹ found on the leaf of nine cucurbit varieties in net cage

Crop	Variety	Number of red pumpkin beetle plant ⁻¹ in net cage
	BARI Misti Kumra-1	2.76 b
Sweet gourd	BARI Misti Kumra-2	2.57 b
	Local Misti Kumra	3.38 a
	BARI Karola-1	0.67 d
Bitter gourd	Taj Karola-88	0.67 d
	Local Karola	0.57 d
	BARI Lau-3	1.81 c
Bottle gourd	BARI Lau-4	1.67 c

Local Lau	2.00 c
Mean	1.79
SE	0.34
LSD value	0.42
Level of significance	0.01
CV (%)	9.99

Means followed by common letter (s) are not significantly different

Table 5. Percent leaf infestation at different stages of three cucurbit crops in the field

	Percentage of leaf infestation		
Crop	Seedling stage	Flowering stage	Fruiting stage
Sweet gourd	33.37 a	14.07 a	13.87 a
Bitter gourd	5.85 c	4.92 c	4.77 c
Bottle gourd	16.69 b	10.52 b	11.11 b
Mean	18.64	9.84	9.92
SE	8.00	2.67	2.70
LSD value	4.58	3.20	4.30
Level of significance	0.01	0.01	0.01
CV (%)	10.30	13.64	18.19

Means followed by common letter (s) are not significantly different

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Table 6. Percentage of leaf infestation found	d among nine cucurbits v	arieties at different stages in the field

		Percentage of leaf infestation		
Crop	Variety	Seedling stage	Flowering stage	Fruiting stage
	BARI Misti Kumra-1	34.76 a	12.78 b	12.18b
Sweet gourd	BARI Misti Kumra-2	28.49 b	13.89 ab	11.94b
	Local Misti Kumra	36.86 a	15.56 a	17.48a
	BARI Karola-1	5.12 e	4.33 d	3.99c
Bitter gourd	Taj Karola-88	6.27 e	4.05 d	4.11c
	Local Karola	6.14 e	6.37 cd	6.19c
	BARI Lau-3	16.37 cd	11.83 b	10.83b
Bottle gourd	BARI Lau-4	14.81 d	7.42 c	10.83b
	Local Lau	18.89 c	12.31 b	11.67
Mean		18.64	9.83	9.91
SE		4.08	1.44	1.46
LSD value		3.32	2.32	-
Level of significance		0.05	0.05	NS
CV (%)		10.30	13.64	18.19

Means followed by common letter (s) are not significantly different

Table 7. Total leaf area damage at seedling stage of three cucurbit crops in net cage

Crop	Leaf area damaged in net cage (mm ²)		
Sweet gourd	686.44 a		
Bitter gourd	39.42 c		
Bottle gourd	365.94 b		
Mean	363.94		
SE	186.78		
LSD value	26.69		
Level of significance	0.01		
CV (%)	3.08		

Means followed by common letter (s) are not significantly different

Crop	Variety	Leaf area (mm ²) damaged in net cage	
	BARI Misti Kumra-1	698.14 b	
Sweet gourd	BARI Misti Kumra-2	623.95 c	
	Local Misti Kumra	737.24 a	
	BARI Karola-1	31.67 f	
Bitter gourd	Taj Karola-88	39.83 f	
	Local Karola	46.75 f	
	BARI Lau-3	355.92 e	
Bottle gourd	BARI Lau-4	338.83 e	
	Local Lau	403.08 d	
Mean		363.93	
SE		94.04	
LSD value		26.69	
Level of significance	e	0.01	
CV (%)		3.08	

Table 8. Total leaf area damage at seedling stage on nine cucurbit varieties in net cage

Means followed by common letter (s) are not significantly different

Table 9. Food consumption of adult on the leaves of three cucurbit crops

	Adult food consumption (mm ²)		
Crop	Total food consumption	Daily food consumption	
Sweet gourd	294.55 a	42.08 a	
Bitter gourd	33.19 c	4.74 c	
Bottle gourd	136.96 b	19.57 b	
Mean	154.90	22.13	
SE	75.98	10.85	
LSD value	25.34	3.62	
Level of significance	0.01	0.01	
CV (%)	6.96	6.96	

Means followed by common letter (s) are not significantly different

Table 10. Food consumption of adult on the leaves of nine cucurbit varietie	Table 10. Food consum	ption of adult of	n the leaves of nin	e cucurbit varieties
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		Adult food consumption (mm ²)	
Crop	Variety	Total food consumption	Daily food consumption
Sweet gourd	BARI Misti Kumra-1	264.00 b	37.71 b
	BARI Misti Kumra-2	283.35 b	40.48 b
	Local Misti Kumra	336.29 a	48.04 a
Bitter gourd	BARI Karola-1	35.75 f	5.11 f
	Taj Karola-88	34.75 f	4.96 f
	Local Karola	29.07 f	4.15 f
Bottle gourd	BARI Lau-3	170.47 c	24.35 c
	BARI Lau-4	105.50 e	15.07 e
	Local Lau	134.92 d	19.27 d
Mean		154.90	22.13
SE		38.88	5.55
LSD value		25.34	3.62
Level of significance		0.01	0.01
CV (%)		6.96	6.96
0.11 1.1	1 ()	1 1.00	

Means followed by common letter (s) are not significantly different

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