Varietal preference and efficacy of various chemicals against red pumpkin beetle (*Aulacophora foveicollis* l.) (Coleoptera: chrysomelidae) in different cucumber (*Cucumis sativus*) cultivars

Inzimam-ul-Haq, Shah Alam Khan and Mehran Ullah

ABSTRACT____ Experiments were conducted to determine the resistance level of different cultivars and study the efficacy of various synthetic insecticides against red pumpkin beetle (*Aulocohphora foveicollis*) at the New Developmental Malakandir Farm, the University of Agriculture Peshawar, during 2018. In the first experimental trial, data were collected on the mean population density of red pumpkin beetle on three cultivars (Desi, Super Green and Madam-560) of cucumber crop. While in the second experiment, three insecticides (Carbaryl, Cypermethrin, Malathion) and an Untreated/Control were used to study the effect of these insecticides on Desi (susceptible) and Madam 560 (resistant) cultivars regarding red pumpkin beetle on cucumber cultivar. Randomized Complete Block Design (RCBD) was used in this experiment. The data on the relevant parameters were recorded at weekly intervals for eleven (1st experiment) and four weeks (2nd experiment) i.e. till termination of the experiments. During first experiment, it was observed that significantly lowest population density of red pumpkin beetle (0.82) per plant and highest yield (8981.3 kg ha^{-1}) was observed from Madam 560, while significantly highest mean population density of red pumpkin beetle (4.06) per plant and lowest yield (7313.7 kg ha^{-1}) was obtained from Desi cultivar. Significantly maximum mean number of red pumpkin beetle plant^{-1} was recorded in the seventh week (29 May) from Desi, Super Green and Madam 560 cultivars with 6.41, 4.50 and 1.62 beetle plant^{-1}, respectively. During the cropping season, mean population density of red pumpkin beetle plant^{-1} reached its peak in the seventh week (29 May). After 7th week, a gradual decline was occurred in the mean population density of red pumpkin beetle plant^{-1}. In second experiment, lowest mean population density of red pumpkin beetle adults plant^{-1} (0.12), area of consumption (3.12 mm^2), percentage weight loss (1.60 %) and highest yield (21555 kg ha^{-1}) was recorded from Madam 560 treated with Carbaryl, while highest mean population density of red pumpkin beetle adult plant^{-1} (7.09), area of consumption (36.11 mm^2), percentage weight loss (17.27%) and lowest yield (7247 kg ha^{-1}) was recorded from untreated Desi cultivar. It was followed by untreated Madam 560 cultivar where 5.40, 25.92 mm^2, 13.20% and 8766 kg ha^{-1}. Madam 560 is significantly better cultivar than Super Green and Desi, as this cultivar results in lowest population density of red pumpkin beetle and highest yield of cucumber fruit. Carbaryl is significantly better as compared to Malathion and Cypermethrin because it reduces the population of red pumpkin beetle.

Keywords: *Aulacophora foveicollis,* Cucumber, Desi, Madam 560, Resistant, Susceptible, Super Green.

INTRODUCTION:

Family cucurbitaceae is one of the most important and biggest group of vegetables; (Khan, 2015), as it is composed of 118 genera and 825 different species of vegetables, cucumber is one of the most important vegetable (Khan *et al*., 2015; Maurya *et al*., 2015). Cucumber is one of the most important vegetables

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wrinkles as well as can be utilized as moisturizer by inhibiting tyrosinase (Murad and Nyc, 2016), as it contains potassium in high amounts (50-80 mg/100g), cucumber can also be useful for the patients of both high and low blood pressures (Kashif *et al*., 2008).

The loss in production is caused by different insect ranges from 30-100% dependent upon the specie of cucumber and weather (Dhillon *et al*., 2005). Cucumber is subjected to damage by different insect pests from the initial stages to the final harvest of the crop. Cucumber and Squash are highly susceptible to red pumpkin beetle (Saljoqi and Khan, 2007). Muskmelon, cucumber and sweet gourd are highly vulnerable to *A. foveicollis* while bitter gourd, sponge gourd and ribbed gourd are non-preferred hosts of red pumpkin beetle (Khan *et al*. 2012). The population of the *A. foveicollis* remains considerably throughout the year except during second fortnight of December till January. The population of *A. foveicollis* increases from February-April and May-October (Khan, 2015). In young shoots and leaves, population density of *A. foveicollis* increases but as the leaves of cucumber crop matures infestation of *A. foveicollis* decreases (Alao *et al*., 2017). *A. foveicollis* damages cucumber at cotyledon stage, as small fruits start to dry up, however, mature fruits maybe not suitable for the consumption of humans (Kamal *et al*., 2014).

The utilization of chemicals such as carbaryl and endosulfan and deep plough can be used to control *A. foveicollis* (Saleem and Shah, 2010). Malathion can be used at various concentrations to control the infestation of *A. foveicollis* (Hassan *et al*., 2011). The use of Carbaryl in combination with need seed kernel extract and yellow sticky trap control can be

used as integrated pest management of *A. foveicollis* (Rashid *et al.*, 2015). Carbaryl, cypermethrin and chlorphyripos are the most effective chemicals which can be used to control the infestation caused by *A. foveicollis* (Ratnakar *et al.*, 2016). The damage caused by these insect pests plays an important role in reducing the quality and quantity of the cucumber crop. Keeping in view the significance of this crop and its associated insect pests, this study is designed to investigate the following objectives:

MATERIALS AND METHODS:

The study was conducted on the screening and management of Red pumpkin beetle (*Aulacophora foveicollis* L.) on cucumber cultivar. This research trial was consisted of two experiments:

Experiment 1: Varietal preference of red pumpkin beetle against cucumber cultivars

An experimental area of 244m² was prepared in Newly Developed Malakandair Farm, The University of Agriculture Peshawar. There were 3 blocks (replications), with 81m² area. In each block there were 3 treatments. Each variety was replicated 3 times in one block. The size of each treatment was 27m². Randomized Complete Block Design (RCBD) was used in this experiment. Three cultivars (Desi, Super Green and Madam-560) of cucumber were sown during last week of March with plant-plant and row-row distance of 40cm and 150cm, respectively. Seeds were sown in lines with two seeds hole^{-1} in each row with four plants in each row. There were three rows in each treatment of every replication. Only one healthy seedling was left while the other one was uprooted. All the agronomic practices were conducted uniformly throughout the experiment.

Parameters:

1. Mean population trend of Red pumpkin beetle (*Aulacophora foveicollis* L.) recorded per plant on three different cucumber cultivars during 2018

Three selected cultivars were sown to observe the population of red pumpkin beetle. The experimental area was kept unsprayed throughout the screening. The data was recorded as soon as the pest appears (17th April) in the field and continued for 11 weeks till 26th June 2018. From each treatment, five vines were selected out of twelve vines and tagged to record the data concerning the population of *A. foveicollis*. The data was recorded at weekly interval throughout the experiment. The scrapped galleries were counted of selected plant leaves (Top, middle and bottom) to observe the damage caused by *A. foveicollis* and average population of pest was calculated (Shinde *et al*. 2018).

Total rows per plot=9
Total plants per plot=36
Total plots=3
Total selected vines=5

2. Yield characteristics of three different cucumber cultivars (Desi, Super Green and Madam 560) during 2018

Cucumber was harvested when full length has been reached. During the peak season, the ripened cucumbers were harvested every day till 30th June. In each treatment ripened fruits were taken in every replication, from each variety. Yield data was recorded in grams with the help of scale and then converted in to kg per hectare (Gruda *et al.*, 2017).

Experiment 2: Efficacy of three different chemicals on cucumber cultivar against red pumpkin beetle:

For this trial an experimental area of 372m² was prepared in Newly Developed Malakandair Farms, The University of Agriculture Peshawar. There were 3 replications. The size of each replication was 124m². In each replication there were 8 treatments. Desi (susceptible) and Madam 560 (resistant) were sown in these blocks during 3rd week of July with plant-plant and row-row distance of 40cm and 150cm, respectively. There were three plants in each row and three rows in each treatment of every replication. The effect of three different chemicals i.e., Cypermethrin, Malathion and Carbaryl was observed on red pumpkin beetle from these two cucumber varieties. A buffer zone of 2 meter was taken to isolate the blocks. Each variety was replicated 3 times in each block. The size of each treatment was 15.5m².

Treatments combination:

- T_1 = Cypermethrin (3.5ml/lit) + Desi
- T_2 = Malathion (4ml/lit) + Desi
- T_3 = Carbaryl (5ml/lit) + Desi
- T_4 = Desi (Control)
- T_5 = Cypermethrin (3.5ml/lit) + Madam 560
- T_6 = Malathion (4ml/lit) + Madam 560
- T_7 = Carbaryl (5ml/lit) + Madam 560
- T_8 = Madam 560 (Control)

Application:

Cypermehtrin (3.5 ml/lit), Carbaryl (5 ml/lit) and Malathion (4 ml/lit) were applied at the start of the infestation of the *A. foveicollis* on cucumber crop. Application of these chemicals was done four times at weekly interval from 12th August 2018 to 2nd September 2018.

Parameters:

1. Adult pumpkin beetles per plant:

During this parameter nine leaves in three representative plants out of total nine plants were selected for recording the data on both side of leaves in each replication and the number of adult red pumpkin beetle were recorded with the help of visual observation. The observation was made in the morning and afternoon. As the beetles are active so the observation was made carefully without disturbing the plant leaves. Data was

taken four times at weekly interval. Data collection was started at 13th August 2018 and continued till 3rd September 2018. Mean number of adult pumpkin beetle per plant was calculated (Khan, 2012).

2. Food consumption of beetles (Graph paper):

The adult of this beetle usually feed on the leaves by creating uneven holes and causes damage to flowers and flower buds (Sarker *et al.*, 2016). Food consumption of the adult beetles was recorded with the help of graph paper. Eight petri dishes were taken and eight leaves from treated and untreated plots were kept in these dishes. Soil was kept in the base of petri dish, one leaf from each cucumber cultivars were placed on the soil in the petri dish. One adult beetle was released in each petri dish. For the prevention of leaf from withering cut end of petiole of each leaf was provided with water-soaked cotton pad. Infested leaves were taken and were put on the graph paper. The infestation was measured through the square millimeter graph paper. After every 24 hours leaf and beetle were replaced. Data of the feeding activity was recorded after every 24 hours. In this manner, data was recorded for 72 hrs (three intervals with 24 hrs-1) on these infested leaves and mean was calculated (Sarker *et al.*, 2016).

3. Food consumption of beetles (Percentage weight loss):

Eight fresh leaves were taken from all treatments and were weighed with the help of electric balance (Initial Weight). These leaves were placed with the beetle inside a petri dish. For the prevention of leaf from withering cut end of petiole of each leaf was provided with water-soaked cotton pad. After 24 hours the infested leaf was taken and again was weighed with the help of electric balance (Final Weight). The final weight was subtracted from initial weight. In this manner data was collected after 24, 48 and 72 hours and mean consumption was calculated. Food consumption was expressed as percentage weight loss. Percentage weight loss was calculated by the following formula (Kamal *et al.*, 2013).

Percentage weight loss= Weight loss of leaf / Total weight of leaf × 100

4. Yield per treatment:

Cucumber was harvested when full length has been reached. During the peak season, the ripened cucumbers were harvested every day. In each treatment ripened fruits were taken in every replication, after the application of chemicals. The data was recorded in grams with the help of scale and then converted in to kg per hectare (Gruda *et al.*, 2017).

Statistical Analysis:

All the recorded data was analyzed statistically by using STATISTIX (8.1) software. Analysis of variance (ANOVA) was constructed and for the differentiation of means, the Least Significant Difference (LSD) test was performed**.**

III. RESULTS

4.1 Mean population trend of Red pumpkin beetle (*Aulacophora foveicollis* L.) per plant during 11 weeks interval on different cultivars of cucumber crop during 2018.

The results regarding the population trend of red pumpkin beetle on different cultivars per plant has been given in table 4.1. It is evident from the results that the infestation of red pumpkin beetle has been recorded on three various cultivars of cucumber crop but there was significant variation in the population density of red pumpkin beetle among all the cultivars and time interval (weeks). The interaction of various cucumber cultivars with time interval (weeks) was also found significant. There was a gradual increase in the population density of red pumpkin beetle from the 1st week (17th April) up to seventh week (29th May). The significantly maximum mean population density was recorded in the seventh week from Desi, Super Green and Madam 560 cultivars with 6.41, 4.50 and 1.62 numbers of beetles per plant, respectively. During the cropping season, mean population density of red pumpkin beetle per plant reached to its peak in the seventh week. After 7th week, a gradual decline was recorded in mean population density of red pumpkin beetle plant-1. The lowest mean population density plant-1 was observed in the 11th week (26th June) from Desi, Super Green and Madam 560 with 1.63, 0.80 and 0.12 numbers of beetles per plant. From week one up to 11th week of the cropping season, the significantly highest mean population density was recorded from Desi cultivar followed by Super Green and Madam 560. The data regarding the pooled mean on the population trend of red pumpkin beetles on three various cultivars exhibited that the significantly maximum mean population density of red pumpkin beetle was observed from Desi cultivar (4.06 beetles per plant) followed by Super Green (2.44 beetles per plant) while the lowest mean population density was observed from Madam 560 (0.82 beetles per plant). It is concluded from the results that among the three cultivars of cucumber the most favorite and preferred host of red pumpkin beetle is Desi, followed by Super Green while Madam 560 was found to be the least preferred host.

Table 4.1Mean population trend of *Aulacophora foveicollis* (L.) observed per plant on three different cucumber cultivars from April to June 2018.

Time interval (Weeks)					Mean
17April	1.64k-n	0.72pqr	0.10s	0.82f	
24April	3.46fgh	1.86klm	0.51qrs	1.95e	
1May	4.48de	1.92kl	0.80pqr	2.40d	
8May	4.96cd	2.13jk	1.01opq	2.70c	
15May	5.35bc	3.67f	1.40mno	3.47b	
22May	5.69b	3.84f	1.20pop	3.58b	
29May	6.41a	4.50de	1.62lmn	4.18a	
5June	4.45e	3.00hi	1.01opq	2.82c	
12June	3.52fg	2.59ij	0.73pqr	2.28d	
19June	3.05ghi	1.86klm	0.49rs	1.80e	
26June	1.63kn	0.80pqr	0.12s	0.85f	
Mean	4.06a	2.44b	0.82c		

Table 4.2   **Yield characteristics of three different cucumber cultivars (Desi, Super Green and Madam 560) during 2018**

Treatments	Yield (kg ha^{-1})
Desi (T$_1$)	7313.7c
Super Green (T$_2$)	7775.0b
Madam 560 (T$_3$)	8981.3a

LSD = 183.09

LSD = (P < 0.05).

4.3   Effect of various chemicals on the mean population density of Red pumpkin beetle (*Aulacophora foveicollis* L.) adults per plant in cucumber crop during 2018

The analysis of variance for mean population density of Red pumpkin beetle adults showed significant influence by various chemicals on two different cucumber cultivars. The results regarding the effect of various chemicals on mean population density of red pumpkin beetle adult plant^{-1} on two cucumber cultivars are given in Table 4.3. Among all the eight treatments utilized, mean values showed significant difference. Significantly highest mean population density of red pumpkin beetle adult plant^{-1} (7.09) was observed from T$_4$ (Desi), followed by T$_8$ (Madam 560) with 5.40 beetles per plant. There was no significant difference on mean population density of red pumpkin beetle per plant between T$_3$ (Carbaryl + Desi) and T$_5$ (Cypermethrin + Madam 560). However, significantly lowest mean population density of red pumpkin beetle per plant (0.12) was observed from T$_7$ (Carbaryl + Madam 560) followed by T$_5$ (Cypermethrin + Madam 560) with mean population density of 0.52 red pumpkin beetle per plant.

4.2   Yield characteristics of three different cucumber cultivars (Desi, Super Green and Madam 560) during 2018

The analysis of variance regarding yield among three different cucumber cultivars (Desi, Super Green and Madam 560) showed significant difference. The results regarding the yield of three different cucumber cultivars are given Table 4.2. The significantly lowest yield (7313.7 kg ha^{-1}) was obtained from Desi cultivar, followed by Super Green cultivar with (7775.0 kg ha^{-1}). However, significantly highest yield (8981.3 kg ha^{-1}) was obtained from the Madam 560 cultivar.

Table 4.3   Effect of three different chemicals on the mean population density of *Aulacophora foveicollis* (L.) adults per plant on two cucumber cultivars during 2018

Chemicals	Mean Population Density per plant	
	Desi	Madam 560
Cypermethrin (3.5ml/lit)	2.38 d	0.52 f
Malathion (4ml/lit)	3.12 c	1.52 e
Carbaryl (5ml/lit)	0.91 f	0.12 g
Control	7.09 a	5.40 b
LSD = 0.3882		

LSD = (P < 0.05).

4.4   Area of consumption (mm²) by the adult of red pumpkin beetle (*Aulacophora foveicollis* L.) fed on leaves two different cucumber cultivars treated with different chemicals under laboratory conditions

Food consumption (mm²) of cucumber leaves of two different cucumber cultivars by adult red pumpkin beetle (*Aulacophora foveicollis* L.) treated with different insecticides is presented in table 4.4. The consumption area was recorded through a graph paper and calculated in mm². The recorded data showed that significantly highest mean consumption area of leaves by the adults of red pumpkin beetle was observed from untreated Desi cultivar leaves followed by untreated Madam 560 cultivar leaves with consumption area of 36.11 and 25.92 mm², respectively. The significantly lowest consumption area was recorded from Madam 560 cultivar leaves treated with Carbaryl (3.12 mm²) followed by Cypermethrin treated Madam 560 cultivar leaves with 6.25 mm² mean consumption area.

Table 4.4   Area of consumption (mm²) by the adult of red pumpkin beetle (*Aulacophora foveicollis* L.) fed on leaves of two different cucumber cultivars treated with different chemicals under laboratory conditions

Chemicals	Mean Consumption (mm²) of leaf per day	
	Desi	Madam 560
Cypermethrin (3.5ml/lit)	13.84 d	6.25 g
Malathion (4ml/lit)	17.80 c	11.52 e
Carbaryl (5ml/lit)	7.49 f	3.12 h
Control	36.11 a	25.92 b
LSD = 0.3323		

LSD = (P < 0.05).

4.5   Mean percentage weight loss of two cucumber cucumber cultivars leaf by consumption of adult red pumpkin beetle (*Aulacophora foveicollis* L.) treated with different chemicals under laboratory conditions

Food consumption in terms of percentage weight loss of two different cultivars of cucumber leaves by the adult of red pumpkin beetle (*Aulacophora foveicollis* L.) treated with different insecticides is presented in table 4.5. The weight loss from leaves was recorded through an electrical balance and expressed in percentage. The recorded data showed that significantly highest mean percentage weight loss of leaf per day by adults of red pumpkin beetle was observed from untreated Desi cultivar leaves followed by untreated Madam 560 cultivar leaves with percentage weight loss of leaf per day of 17.27 and 13.20%, respectively. The significantly lowest mean percentage weight loss of leaf per day was recorded from Madam 560 cultivar leaves treated with Carbaryl (1.60%) followed by Cypermethrin treated Madam 560 cultivar leaves with 2.76% mean percentage weight loss of leaf per day.

Table 4.5   Percentage weight loss of two cucumber cultivars leaf by the consumption of adult red pumpkin beetle (*Aulocohpora foveicollis* L.) treated with different chemicals under laboratory conditions

Chemicals	Mean Percentage weight loss of leaf per day	
	Desi	Madam 560
Cypermethrin (3.5ml/lit)	7.51 d	2.76 g
Malathion (4ml/lit)	9.37 c	5.41 e
Carbaryl (5ml/lit)	4.05 f	1.60 h
Control	17.27 a	13.20 b
LSD = 0.9312		

LSD = (P < 0.05).

4.6   Effect of various chemicals on the yield of cucumber crop during 2018.

The analysis of variance for yield showed significant effect by the application of various chemicals on two different cucumber cultivars. The results regarding the effect of various chemicals on the yield of two different cucumber cultivars are given Table 4.6. Significantly lowest mean yield (7247 kg ha^{-1}) was observed from T$_4$ (Desi), followed by T$_8$ (Madam 560) with 8766 kg ha^{-1}. However, significantly highest mean yield (21555 kg ha^{-1}) was observed from T$_7$ (Carbaryl + Madam 560) followed by T$_5$ (Cypermethrin + Madam 560) with mean yield of 18305 kg ha^{-1}.

Table 4.6   Effect of various chemicals on the yield of two cucumber cultivars during 2018

Chemicals	Yield (kg ha^{-1})	
	Desi	**Madam 560**
Cypermethrin (3.5ml/lit)	13071 e	18305 b
Malathion (4ml/lit)	10944 f	14894 d
Carbaryl (5ml/lit)	15695 c	21555 a
Control	7247 h	8766 g
LSD = 731.44		

LSD = ($P < 0.05$).

IV. DISCUSSION

Our data regarding the population trend of red pumpkin beetle per plant are in conformity with Khan *et al.* (2015) who stated that the population of red pumpkin beetle starts gradually increasing in the last week of April and reached to its peak in the 3rd week of May, and after 5th week the population of red pumpkin beetle starts decreasing gradually. The same results were also mentioned by Saljoqi and Khan (2007) who recorded significantly highest number of red pumpkin beetle in the mid of May. Al-Ali *et al.* (1982) also reported that the infestation of red pumpkin beetle was severe in the month of April but decreased in the following months. Khan (2015) also stated that the environmental conditions of Pakistan is best suited for red pumpkin beetle as most of the vegetables are grown in tropical and sub-tropical climatic conditions that's why cucumber is one of the most important host for red pumpkin beetle in Pakistan.

Our results regarding the use of chemicals are in conformity with Ratnakar *et al.* (2016) who studied different insecticides comparatively and concluded that Carbaryl is the most effective against red pumpkin beetle followed by Cypermethrin and Malathion. Saleem and Shah (2010) also recommended the use of Carbaryl as the best control measure

red pumpkin beetle. The same results were also mentioned by Mehmood *et al.* (2006) who stated that Carbaryl is the most effective chemicals against the adult of red pumpkin beetle in cucumber crop. Rathod *et al.* (2009) stated that the Carbaryl and Cypermethrin has the potential to control red pumpkin beetle in cucumber crop. Singh and Gupta (1970) also explored that the use of Malathion at the rate of 1000 ha^{-1} can control red pumpkin beetle.

There was contradiction regarding the efficacy of Carbaryl against red pumpkin beetle as, Dabi *et al.* (1980) used 14 different insecticides against red pumpkin beetle and find out that 64% reduction was observed in the population of red pumpkin beetle in Phosphamidon treated plots even after 15 days of treatments application followed by Carbaryl and Endosulfan. However, Singh and Gill (1982) reported that Carbaryl killed highest mean number of red pumpkin beetles plant^{-1} (44.3) and was most effective chemical in controlling the infestation caused by red pumpkin beetle. Khan and Jehangir (2000) also used three different concentrations (0.5, 1.0 and 2.0%) of Carbaryl against red pumpkin beetle and concluded that Carbaryl shows better control when used at high concentrations, however at lower and safe concentrations it was effective against red pumpkin beetle even after 7 days. Rashid *et al.*, (2015) stated that Carbaryl in combination with neem seed extract and yellow sticky traps are found to be the best control method against red pumpkin beetle in different ecological regions (Faisalabad, Sargodha, Multan) of Pakistan.

Adnan *et al.*, (2019) also stated that Carbaryl was found to be the most effective insecticide against red pumpkin beetle followed by Karate while Hostathion was found to be the best acaricide against two spotted spider mites. Brown (2003) also stated that Carbaryl was the most effective chemical used against red pumpkin beetle in comparison with trichlorofon and control. Our results regarding the mean consumption (mm^2) are in line with Hassan *et al.*, (2012) who stated that average consumption of 15.87 mm^2 was recorded from leaves infested by red pumpkin beetle under laboratory conditions. Our results were also in conformity with Kamal *et al.*, (2014) who recorded lowest total leaf area consumption on untreated bitter gourd (39.42 mm^2) while highest was recorded on sweet gourd with 686.44 mm^2. Our results are also in line with Hassan *et al.*, (2011) studied effect of malathion on mean consumption areas of cucurbitaceous leaf and found that the mean consumption on after 24 hours is 4.20 mm^2 from malathion treated leaf.

CONCLUSIONS AND RECOMMENDATIONS

Madam 560 is significantly better cultivar than Super Green and Desi, as this cultivar results in lowest population density of red pumpkin beetle and highest yield of cucumber fruit.

Carbaryl is significantly better as compared to Malathion and Cypermethrin because it reduces the population of red pumpkin beetle. Further, this insecticide results in lowest mean consumption (mm²) per day, lowest percentage weight loss of leaf per day and significantly highest cucumber yield. Madam 560 is recommended as better cucumber cultivar against red pumpkin beetle.

Author's contribution: Inzimam-Ul-Haq conducted the experiment, **Mehran Ullah** analyzed data and wrote the manuscript and **Shah Alam Khan** designed the experiment and language checked.

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