Plant Protection, 09 (01) 2025. 31-42 DOI:10.33804/pp.009.01.5359

Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

Research Article

QUANTITATIVE ANALYSIS OF BIOCHEMICAL TRAITS IN CUCURBITS AND THEIR ASSOCIATION WITH FIELD RESISTANCE TO INSECT PESTS

[a]Abdul Latif, [b]Taimur Khan, [b]Abdul Ahad, [c]Sidra Iqbal, [d]Majid Karim, [e]Mohammad Shafiq u Rahman, [f]Ikram-ul-Haq, [g]Ahmed Raza

[a] Insect Pest Management Program, Institute of Plant and Environmental Protection, National Agriculture Research Centre, Pakistan Agricultural Research Council, Islamabad, 45500, Pakistan.
[b] Department of Entomology, The University of Agriculture, Peshawar, 25130, Pakistan.
[c] Department of Plant Breeding & Genetics, University of Agriculture, Faisalabad, Constituent College Depalpur, Okara, Pakistan.
[d] Crop Diseases Research Institute, Sub Station Murree, Ministry of National Food Security and Research, Islamabad, Pakistan.
[e] Department of Plant Breeding and Genetics, College of Agriculture, University of Sargodha, 40100, Sargodha, Pakistan.
[f] Department of Plant Pathology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, 63100, Bahawalpur, Pakistan.
[g] Plant Virology Lab, Crop Diseases Research Institute, National Agriculture Research Centre, Pakistan Agricultural Research Council, Islamabad, 45500, Pakistan.

ARTICLE INFO

Article history
Received: 27th August, 2024
Revised: 20th November, 2024
Accepted: 20th January, 2025

Keywords
Cucurbitaceae
Aulacophora foveicollis
Bactrocera cucurbitae
Proximate analysis
Correlation
Pest management strategies

ABSTRACT

Developing sustainable pest management strategies that reduce reliance on synthetic pesticides requires an understanding of the complex interactions between plant biochemistry and pest response. This study aimed to evaluate the biochemical composition of six cucurbitaceous plant species, cucumber, pumpkin, bitter gourd, bottle gourd, squash, and sponge gourd, by analyzing their moisture, ash, protein, fat, and fiber content and investigating potential correlations between these biochemical factors and insect pest infestation, specifically *Aulacophora foveicollis* L. and *Bactrocera cucurbitae* C. populations. Field experiments revealed that *A. foveicollis* primarily preferred sponge gourd as its host, while bitter gourd remained uninfested. In contrast, *B. cucurbitae* exhibited a preference for bitter gourd among the tested cucurbitaceous species. Proximate analysis showed that cucumber had the highest moisture content (94.50%), while pumpkin had the highest ash content (0.80%). Squash contained the highest crude protein (1.80%), whereas bitter gourd had the highest crude fiber (1.04%). The highest crude fat content (0.63%) was recorded in pumpkin. Correlation analysis indicated that *A. foveicollis* was positively correlated with moisture, fat, and protein but negatively correlated with fiber and ash. Similarly, *B. cucurbitae* showed a positive correlation with moisture and protein but a negative correlation with fat, fiber, and ash. This study concludes that cucurbits exhibit varying levels of susceptibility to insect pests, and infestation can be influenced by the biochemical composition of the plant. These findings highlight the importance of understanding plant biochemistry for developing effective pest management strategies.

Corresponding Author: Ahmed Raza
Email: ahmedraza7128@parc.gov.pk

INTRODUCTION

The Cucurbitaceae family encompasses a wide variety of crops commonly referred to as cucurbits. These crops are staples in the human diet, forming the basis of many popular dishes (Grumet et al., 2021). This diverse family includes *Cucumis sativus* L., *Momordica charantia* L., *Praecitrullus fistulosus*, *Lagenaria siceraria* (Molina), *Luffa aegyptiaca* Mill., and *Cucurbita maxima*, among others. Depending on personal preference, these crops can be consumed fresh (seeds), processed for oil, utilized in medicine (Dhillon et al., 2017), or primarily used as vegetables in cooking (Ajuru and Nmom, 2017).

This family is notably diverse, comprising approximately 120 genera and 1,000 species, thriving best in humid and sub-humid climates (He, 2023). In Vietnam alone, there are 23 genera and 53 species of cucurbits (Akdura and Culal-Kilic, 2022), a situation that is quite similar in Pakistan. These crops are rich in essential vitamins and minerals, making them an integral part of a healthy diet (Baloglu, 2018).

Cucurbits are affected by several insect pests. Among these, *Aulacophora foveicollis* L. and *Bactrocera cucurbitae* are widespread across Asia, Africa, Europe, and Australia, causing significant damage to these crops (CABI, 2020). Although *A. foveicollis* L. feeds on all cucurbits, its primary hosts include pumpkin, bottle gourd, and muskmelon (Ahmad et al., 2020), with damage ranging from 35% to 70% (Regmi and Paudel, 2020) and potentially reaching 100%, depending on the season and species.

B. cucurbitae (Coquillett), a member of Diptera (Tephritidae), is found throughout temperate, tropical, and subtropical regions (Sapkota et al., 2013; Hafsi et al., 2015). It has 81 different host plants (Haldhar et al., 2022) and causes significant economic losses to cucurbits, ranging from 30% to 100% (Rai et al., 2014). Infestation renders the fruits unsuitable for consumption or sale (Mondal et al., 2020). This pest prefers to infest young, green, and soft-skinned fruits (Subedi et al., 2021).

Currently, the primary method of pest control is the non-selective use of insecticides, which has led to several adverse effects, including secondary pest outbreaks, pest resistance, and health hazards. The search for alternative and eco-friendly pest control methods has been ongoing. Biological control programs, along with cultural, physical, and genetic control techniques, are among the approaches explored for the benefit of farmers (Piñero et al., 2015). However, the successful integration of these control components into a comprehensive pest management strategy requires greater variability to enhance defense mechanisms. Moreover, accurate information on the occurrence and population dynamics of pests across different hosts under varying agro-climatic conditions is essential (War et al., 2012; Hendrichs et al., 2021).

Plant resistance is a multifaceted and long-term phenomenon for insect control, influenced by various factors (Fahad et al., 2021). Among these, the biochemical properties of plants play a crucial role in defending against insect attacks through the production of secondary metabolites that repel or deter pests (Haldhar et al., 2013; Kaur et al., 2015). These biochemical factors enhance the phenotypic plasticity of plant organs, making them less suitable for insect feeding (Karban, 2011; Li et al., 2023).

Biochemical resistance not only reduces the reliance on insecticides and minimizes environmental harm but also directly impacts the growth and development of insects (Kariyat et al., 2013; Siddiqui et al., 2023). Advances in high-throughput techniques, such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and enzyme activity assays, have significantly improved the ability to quantify these traits.

In the Cucurbitaceae family, a wide array of plant biochemical defense mechanisms is involved, and these mechanisms exhibit varying effectiveness against different pests within the host family. Plant biochemical defense mechanisms are diverse, dynamic, and can influence pest interactions through both direct and indirect pathways (Abd El-Karim et al., 2024).

Recent advancements in genomic tools, such as transcriptomics and metabolomics, have provided valuable understandings for the molecular mechanisms underlying host plant resistance (Xue et al., 2022). Transcriptomic analysis facilitates the identification of differentially expressed genes involved in defense pathways, while metabolomics enables the profiling of a wide range of metabolites associated with plant-insect interactions (Sajeevan et al., 2023). These integrated approaches offer a comprehensive understanding of the biochemical pathways contributing to insect resistance.

Several studies have reported associations between specific biochemical traits and field resistance in cucurbits. Secondary metabolites, including terpenes, phenolics, and alkaloids, are produced in higher amounts in response to insect attacks (Haldhar et al., 2018). Similarly, elevated levels of cucurbitacin compounds have been correlated with reduced pest feeding and oviposition, indicating their potential role in resistance. Enhanced activity of defense-related enzymes has also been linked to increased resistance against insect pests.

However, comprehensive studies involving multiple cucurbit species and pest interactions are needed to validate these associations and identify robust resistance markers. Quantitative analysis of biochemical traits in cucurbits provides valuable understandings for the molecular mechanisms underlying host plant resistance to insect pests. These traits, including secondary metabolites and defense-related enzymes, contribute significantly to the defense response of plant and can serve as potential markers for selecting resistant cultivars.

By understanding the association between biochemical traits and field resistance, researchers can develop sustainable pest management strategies that minimize reliance on chemical pesticides, thereby promoting environmentally friendly and economically viable cucumber production.

The present study was designed to address the gap in existing knowledge regarding the biochemical resistance traits of cucurbits against *A. foveicollis* and *B. cucurbitae*. The objective was to provide insights into the host preference (antixenosis) of these insect pests for different cucurbit species and to analyze the chemical composition of various cucurbits. The findings aim to facilitate the development of effective pest management strategies and support the selection of suitable crops for improved resistance.

MATERIALS AND METHODS

Study site and design

The study was conducted in Yar Hussain, Swabi, Khyber Pakhtunkhwa (KPK), Pakistan, in 2017 to investigate the host preference of various insect pests of cucurbits and their correlation with the proximate composition of different cucurbit species. The research site is located at latitude 34°10’1.34” N and longitude 72°16’40.18” E. The experiment was designed as a randomized complete block design (RCBD) with three replications and six treatments, comprising bitter gourd, sponge gourd, cucumber, bottle gourd, pumpkin, and squash. Each plot had a size of 310 m^2, and uniform agronomic practices were applied across all treatments. No pesticides were applied to any of the plots. Fruit and pest infestations were monitored and recorded weekly until harvest. The collected data were analyzed statistically.

Parameters studied

The study involved the evaluation of various parameters to achieve the research objectives. The following parameters were investigated during the experiment:

Percentage damage by fruit fly

During the study, ripe fruits from each plot were collected. Visibly damaged fruits were separated from the undamaged ones, and the percentage of fruit damage caused by fruit flies was calculated using the following formula:

$$\text{Percent damage } = \frac{\text{Number of damaged fruits}}{\text{Total number of fruits}} \times 100$$

Population density of *A. foveicollis*

The density of the red pumpkin beetle was determined by counting the number of beetles on three randomly selected plants within each plot or treatment at weekly intervals, following the method described by Khan et al. (2015).

Analysis of proximate composition of cucurbits

The proximate composition of cucurbits was analyzed to determine their total moisture, ash, protein, fat, and fiber contents. Fruit samples were collected from the field, and the specified parameters were analyzed in the Chemistry Laboratory at The University of Agriculture, Peshawar.

Sample preparation

The collected samples were cleaned and air-dried upon arrival to prepare them for chemical analysis. A portion of the samples was used to measure moisture content, while the remaining samples were oven-dried at 70°C, with the temperature gradually increased to 100°C to ensure complete drying. The dried samples were then stored for subsequent analysis.

Proximate composition analysis

The fruit samples of cucurbits were subjected to standard proximate analysis to determine their moisture, crude protein, crude fat, ash, and crude fiber contents, following the methods outlined by AACC (2004). The analyses of fiber, fat, protein, ash, and moisture contents were conducted at 3-6-week intervals to ensure accuracy.

Determination of moisture content

The moisture content was determined by weighing the sample and placing it in an oven at 105°C until a constant weight was achieved.

$$\text{Moisture content (\%) } = \frac{\text{W1} - \text{W2}}{\text{Weight of the sample}} \times 100$$

W1 = weight of sample and petri dish
W2 = weight afterward drying
W3 = Sample weight (one gram)

Determination of crude protein

The protein analysis involved three main steps. In the first step, digestion was carried out using copper sulfate, potassium sulfate, and sulfuric acid (H_2SO_4) at varying concentrations. The second step involved distillation using hydrochloric acid (HCl), sodium hydroxide (NaOH), and boric acid in different amounts. This was followed by heating the sample. In the third step, titration was performed using an HCl solution to obtain the final result. The percentage of crude protein was calculated using the following formula, as described by AOAC (1990).

$$\text{Cp content (\%)} = \frac{(S - B) \times 0.1\ N \times 0.014 \times 100}{\text{Weight of the sample} \times V} \times 100$$

Determination of crude fat

The crude fat content was determined by adding petroleum ether to the sample and processing it using Soxhlet apparatus.

$$\text{Crude fat content (\%)} = \frac{W1 - W2}{\text{Weight of the sample}} \times 100$$

Determination of ash content

The ash content was determined using the method described by AACC (2004). The procedure involved taking a well-dried 1-g sample, placing it in a petri dish, and burning it in a furnace. The weight of the sample was recorded both before and after the burning process. The furnace temperature was maintained at 500℃. The ash content was calculated using the following formula:

$$\text{\% Ash} = \frac{W1 - W2}{\text{Weight of the sample}} \times 100$$

Determination of crude fiber

The fiber analysis involved placing 2 g of the sample in a beaker containing 200 ml of 4% sodium hydroxide (NaOH) solution. The mixture was boiled, and the sample was filtered using a woolen cloth. The filtered sample was then immersed in 200 ml of 4% hydrochloric acid (HCl) solution and dried at 100°C. Afterward, it was filtered again and dried for four hours in an oven. The percentage of crude fiber was calculated using the following formula:

$$\text{Crude fat content (\%)} = \frac{W1 - W2}{\text{Weight of the sample}} \times 100$$

Where W1 = Weight of oven dried residue
W2 = Weight after ignition

Statistical analysis

Each content determination experiment was performed in triplicate, and the mean values were calculated to represent the results. Statistical analyses, including Analysis of Variance (ANOVA) and correlation analysis, were conducted using MSTATC software for Windows. Differences were considered statistically significant at $P < 0.05$ for both ANOVA and correlation analysis. To compare mean differences, LSD's New Multiple Range Test was applied, as described by Steel and Torrie (1980).

RESULTS

Seasonal infestation patterns of red pumpkin beetles in cucurbits

The results obtained from the first and second data records, presented in Table 1 and Figure 1, show that red pumpkin beetle infestation was observed throughout the cropping season in all major cucurbits, except bitter gourd. The highest infestation during these observations was recorded on sponge gourd at 0.88 beetles per plant, while bitter gourd had no infestation.

Interestingly, the third observation revealed maximum infestation on squash at 1.56 beetles per plant, followed by cucumber, sponge gourd, and pumpkin, with 0.67, 0.56, and 0.34 beetles per plant, respectively. In the fourth observation, the highest infestation was found on sponge gourd at 0.45 beetles per plant, followed by pumpkin (0.23), squash (0.12), and cucumber (0.12).

In the fifth observation, cucumber hosted the highest number of beetles per plant at 1.23, followed by pumpkin (0.67), squash (0.12), and sponge gourd (0.12). During the sixth, seventh, eighth, and ninth observations, sponge gourd consistently hosted the highest number of beetles per plant, peaking at 1.11. Statistical analysis revealed significant differences in the red pumpkin beetle population among the different cucurbits.

In the tenth observation, cucumbers were most heavily infested, with 1.34 beetles per plant, followed by sponge gourd (1.23), pumpkin (1.22), and squash (0.89). Surprisingly, in the eleventh observation, bottle gourd recorded the highest infestation, with 1.45 beetles per plant. This was followed by sponge gourd (0.89), squash (0.78), pumpkin (0.67), and cucumber (0.56).

Seasonal mean data indicated that sponge gourd was the most preferred host for red pumpkin beetles, with an average infestation of 3.59 beetles per plant from April 19th to June 28th. Cucumber, squash, and pumpkin recorded average beetle counts of 2.70, 2.69, and 2.06 beetles per plant, respectively. Bitter gourd exhibited no beetle infestation throughout the cropping season, while bottle gourd had negligible infestation.

The first observation documented the lowest infestation levels, while the highest infestations occurred during the eighth to tenth observations. Overall, significant differences in red pumpkin beetle populations were observed among the cucurbit crops included in this study.

Table 1: Red pumpkin beetle population plant^{-1} on different cucurbits.

Treatment	April 2017		May 2017					June 2017				Mean pop*
	W3	W4	W1	W2	W3	W4	W5	W1	W2	W3	W4	
Cucumber	0.00^{b}	0.77^{ab}	0.67^{ab}	0.12^{a}	1.23^{a}	0.55^{b}	0.77^{b}	1.00^{b}	1.10^{ab}	1.34^{a}	0.56^{bc}	2.70^{ab}
Pumpkin	0.00^{b}	0.55^{bc}	0.34^{ab}	0.23^{ab}	0.67^{ab}	0.44^{b}	0.44^{bc}	1.10^{b}	0.55^{bc}	1.23^{ab}	0.67^{bc}	2.06^{bc}
Bitter gourd	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{d}
Bottle gourd	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	1.45^{a}	0.011^{cd}
Squash	0.77^{a}	0.55^{bc}	1.56^{a}	0.11^{b}	0.12^{b}	0.44^{b}	0.77^{b}	0.88^{b}	1.21^{ab}	0.89^{ab}	0.78^{abc}	2.69^{ab}
Sponge gourd	0.88^{a}	1.11^{a}	0.56^{ab}	0.45^{c}	0.12^{b}	1.11^{a}	1.33^{a}	1.77^{a}	1.33^{a}	1.22^{ab}	0.89^{ab}	3.59^{a}
LSD (0.05)	0.40	0.55	1.32	0.48	0.77	0.42	0.50	0.44	0.67	0.41	0.77	0.62

*Mean in columns followed by different letters indicates significant at 5% level of probability.

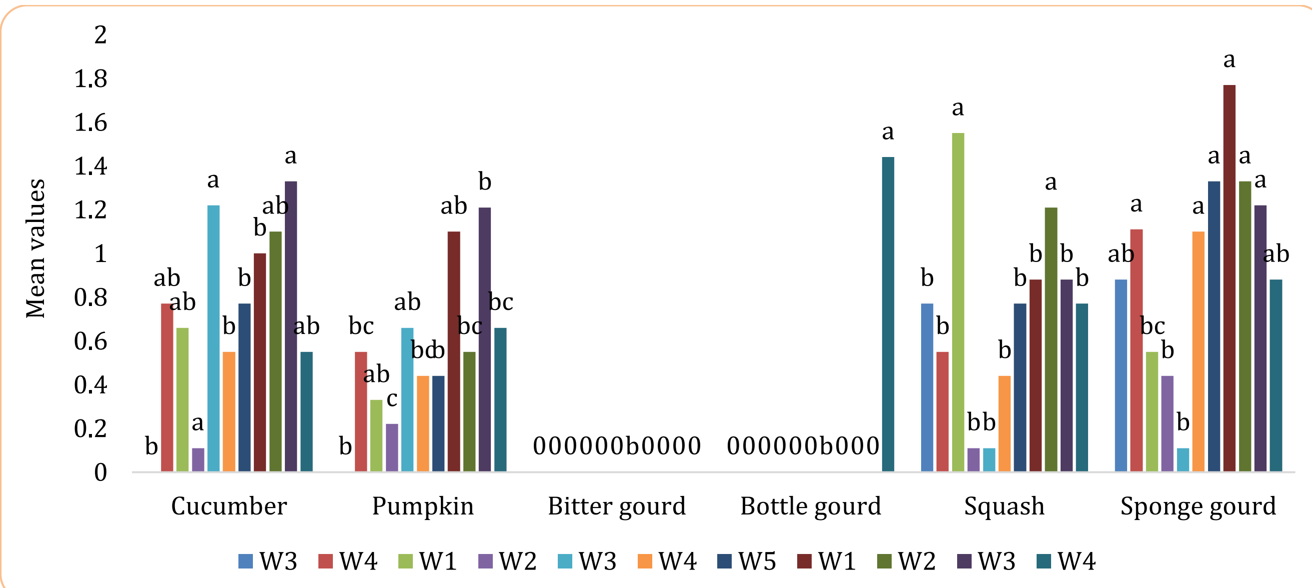

Figure 1. Infestation of red pumpkin beetle on different cucurbit cultivars over 11 weeks. Different lowercase letters indicate significant differences (One-Way ANOVA; means were compared using the LSD test at P ≤ 0.05). The values represent the mean incidence, and the error bars indicate the standard error (± SE) of the replications.

Seasonal trends and host preference of fruit fly damage in cucurbits

During the first and second observations, as shown in Table 2 and Figure 2, the highest fruit fly damage was recorded on bitter gourd, accounting for 14.81% of the total damage. Squash and cucumber followed closely behind. Interestingly, sponge gourd exhibited the highest damage during the third, fourth, fifth, sixth, and seventh observations. Statistical analysis revealed that sponge gourd, cucumber, and squash experienced significantly greater damage compared to the other cucurbits.

In the eighth observation, squash suffered additional fruit fly damage, with a rate of 23.30%. Sponge gourd, bitter gourd, cucumber, pumpkin, and bottle gourd also sustained damage, with rates of 17.76%, 17.36%, 17.29%, 7.41%, and 6.04%, respectively. Bitter gourd was particularly vulnerable to fruit fly damage, with a rate of 8.64%. This was followed by sponge gourd and bottle gourd, which recorded damage rates of 3.41% and 3.18%, respectively. Squash, pumpkin, and cucumber escaped infestation during this observation.

The data from the ninth observation indicated that no fruit fly damage was recorded on any crop. Overall, fruit fly damage began in the last days of April, reaching its peak in the third week of May. The infestation levels fluctuated slightly until the seventh picking on June 7th, after which they declined and reached a minimum by June 21st 2017.

Seasonal population data suggest that bitter gourd emerged as the most preferred host for fruit flies, with a damage rate of 6.06%. Sponge gourd, squash, cucumber, and pumpkin followed with damage rates of 5.15%, 5.02%, 4.60%, and 2.34%, respectively. Notably, bottle gourd demonstrated a degree of resistance to fruit flies compared to the other cucurbits.

Table: 2. Percent damage of fruit fly picking^{-1} on different cucurbits.

Treatments	April	May 2017					June 2017			% Damage
	Picking	Picking	Picking	Picking	Picking	Picking	Picking	Picking	Picking	
Cucumber	3.74^{ab}	14.85^{a}	22.57^{a}	26.72^{ab}	19.77^{a}	20.39^{ab}	20.46^{abc}	17.29^{a}	0.00^{b}	4.60^{a}
Pumpkin	0.00^{b}	0.00^{b}	14.81^{a}	7.40^{c}	3.70^{c}	15.01^{b}	18.51^{bc}	7.41^{b}	0.00^{b}	2.34^{b}
Bitter gourd	14.81^{a}	17.72^{a}	20.02^{a}	25.10^{ab}	18.54^{ab}	16.98^{ab}	23.96^{ab}	17.36^{a}	8.64^{a}	6.06^{a}
Bottle gourd	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{d}	11.81^{b}	11.44^{b}	11.89^{c}	6.04^{b}	3.18^{b}	1.64^{b}
Squash	12.37^{a}	8.59^{ab}	21.68^{a}	24.01^{b}	23.14^{a}	16.87^{ab}	23.59^{ab}	23.30^{a}	0.00^{b}	5.02^{a}
Sponge gourd	0.00^{b}	0.00^{b}	26.45^{a}	30.44^{a}	24.41^{a}	25.81^{a}	29.17^{a}	17.76^{a}	3.41^{ab}	5.15^{a}
LSD (0.05)	11.66	9.97	12.58	6.04	7.21	9.65	9.11	7.20	5.39	0.96

Mean in columns followed by different letters indicate significant at 5% level of probability.

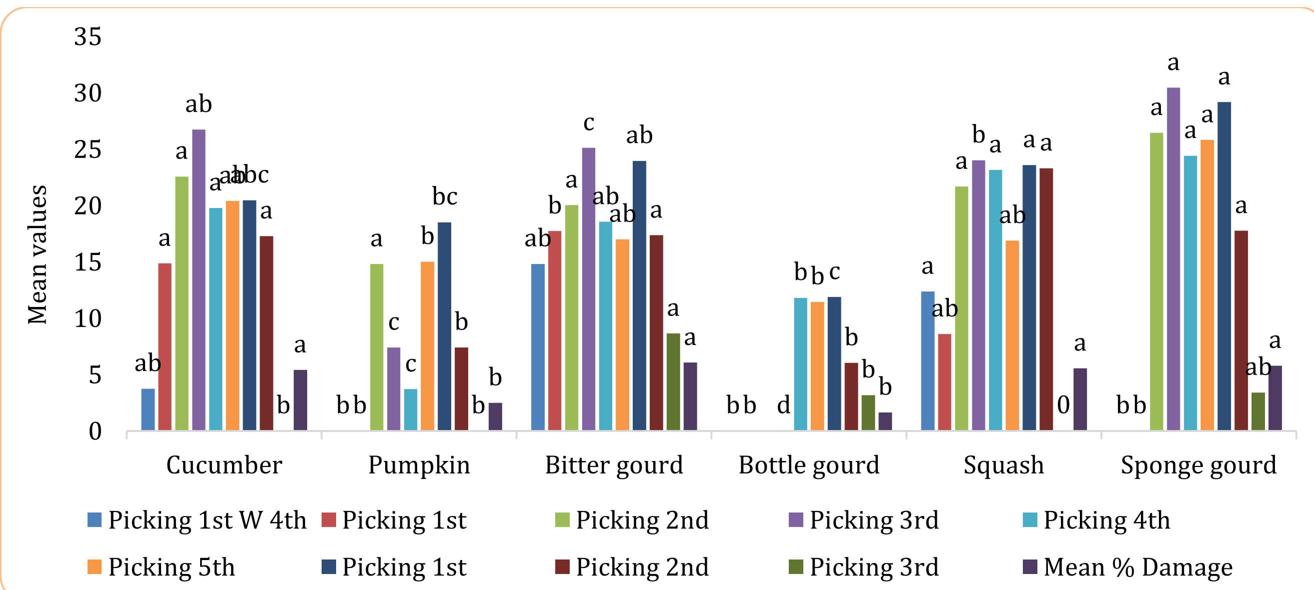

Figure 2. Percentage damage caused by fruit flies on different cucurbit cultivars during the cropping season. Different lowercase letters indicate significant differences (One-Way ANOVA; means were compared using the LSD test at P ≤ 0.05). The values represent the mean percentage damage. Error bars in the figure indicate the standard error (± SE) of the replicates.

Proximate analysis

The proximate analysis includes the amounts of ash, protein, fat, fiber, and moisture content in selected vegetables. Table 3 presents the results of this analysis, revealing noteworthy findings (Figure 3). Pumpkin contained the highest ash content (0.80%), followed by bitter gourd, squash, cucumber, bottle gourd, and sponge gourd, with values of 0.70%, 0.60%, 0.50%, 0.50%, and 0.40%, respectively.

In terms of protein content, squash exhibited the highest level (1.80%), followed by pumpkin (1.33%), bottle gourd (1.13%), bitter gourd (1.09%), sponge gourd

(1.01%), and cucumber (0.86%). These differences were statistically significant.

Pumpkin also had the highest fat content (0.63%), with squash, bitter gourd, bottle gourd, sponge gourd, and cucumber following at 0.62%, 0.20%, 0.19%, 0.19%, and 0.09%, respectively. Bitter gourd showed the highest fiber content (1.04%), followed by pumpkin (0.80%), bottle gourd (0.76%), sponge gourd (0.73%), squash (0.60%), and cucumber (0.50%). Lastly, moisture content was highest in cucumber (94.51%), followed by sponge gourd (93.87%), bottle gourd (93.37%), squash (92.46%), bitter gourd (92.45%), and pumpkin (85.54%).

The data in Table 4 reveal that *A. foveicollis* has a positive correlation with moisture, fat, and protein, while showing a negative correlation with fiber and ash. Similarly, *B. cucurbitae* exhibits a positive correlation with moisture and protein, but a negative correlation with fat, fiber, and ash.

Table 3. Proximate analysis of different cucurbits

Treatments	Ash (%)	Protein (g)	Fat (g)	Fiber (g)	Moisture (%)
Cucumber	0.50^{cd}	0.86^{e}	0.09^{b}	0.50^{c}	94.51^{a}
Pumpkin	0.80^{a}	1.33^{b}	0.63^{a}	0.80^{b}	85.54^{e}
Bitter gourd	0.70^{ab}	1.09^{cd}	0.20^{b}	1.04^{a}	92.45^{d}
Bottle gourd	0.50^{cd}	1.13^{c}	0.19^{b}	0.76^{b}	93.37^{c}
Squash	0.60^{bc}	1.80^{a}	0.62^{a}	0.60^{c}	92.46^{d}
Sponge gourd	0.40^{d}	1.01^{d}	0.19^{b}	0.73^{b}	93.87^{b}
LSD (0.05)	0.19	0.10	0.34	0.12	0.40

Mean in columns followed by different letters indicate significant at 5% level of probability.

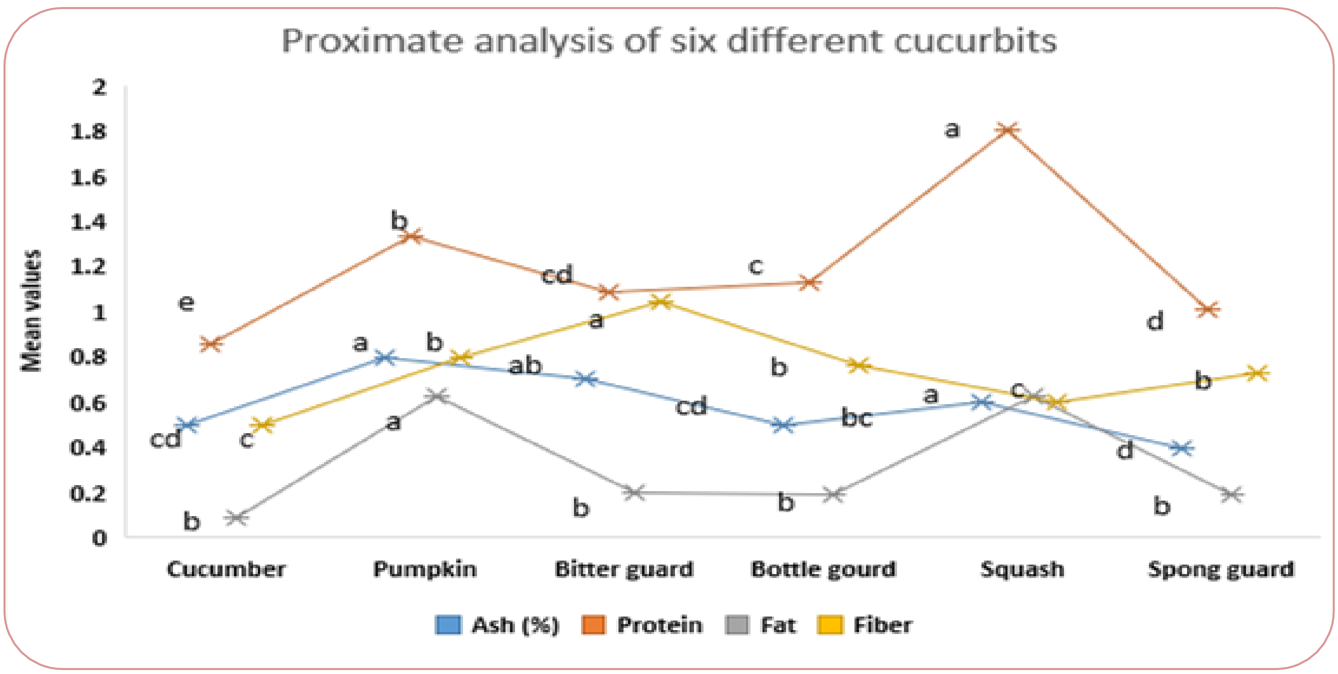

Figure 3. Chemical analysis of selected cucurbit vegetables. Different lowercase letters indicate significant differences (One-Way ANOVA, means compared using the LSD test, P ≤ 0.05). Values represent the sample means. Error bars shown in the figure represent the (± SE) of the replications.

Table: 4. Correlation between cucurbit insect/pests with various biochemical plant factors.

Insect Pests	Ash	Protein	Moisture	Fat	Fiber
Red pumpkin beetle	-0.435^{ns}	0.077^{ns}	0.025^{ns}	0.179^{ns}	-0.689^{ns}
Fruit fly	-0.220^{ns}	0.004^{ns}	0.476^{ns}	-0.186^{ns}	-0.186^{ns}

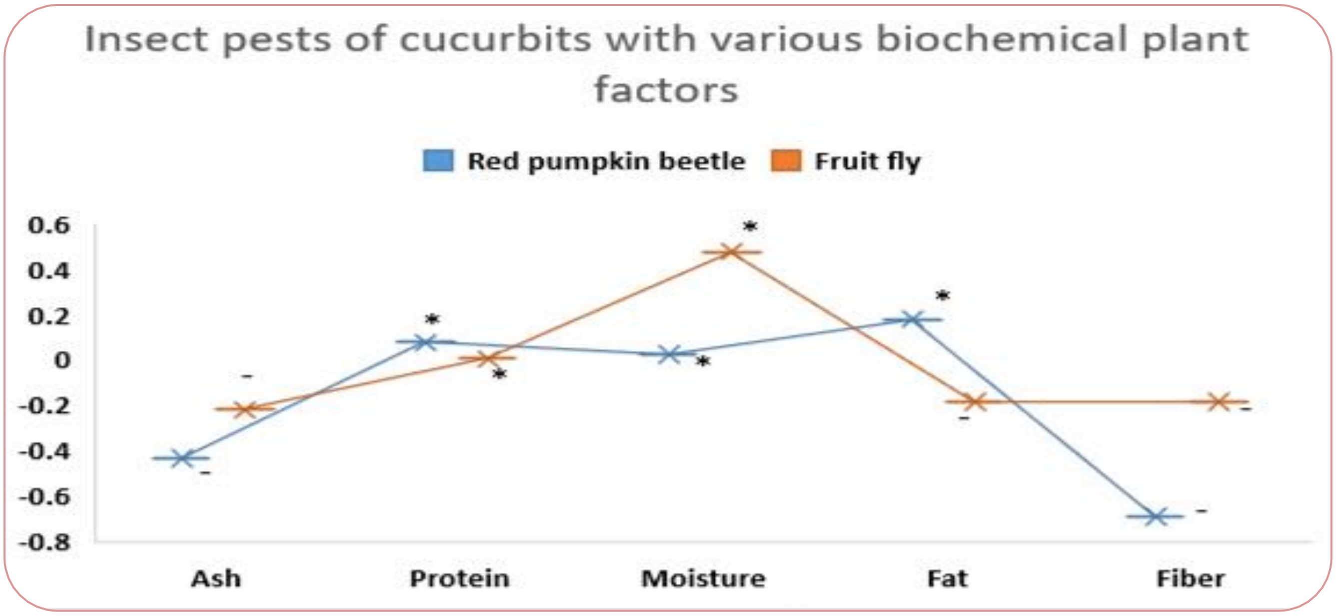

Figure 4. Correlation of insect pests with biochemical factors. (*) represents a positive correlation, while (-) represents a negative correlation.

DISSCUSSION

Biochemistry plays a crucial role, particularly in addressing stress-related issues in plants. The attack of insect pests disrupts normal physiological functions by interfering with one or multiple biochemical processes. Like other plants, cucurbits are sessile, while insects have the ability to move and act as pests of cucurbits. Therefore, studying the biochemical changes that occur between two treatments, healthy cucurbits and those infested by insect pests, is essential.

This study indicates that the highest number of red pumpkin beetles was observed on sponge gourd, with cucumber being the second most infested crop. Interestingly, no infestation was recorded on bitter gourd, while minimal infestation was observed on bottle gourd. Khan (2015) also reported a higher level of infestation on sponge gourd, with two varieties of bitter gourd showing almost no infestation (Khan et al., 2015; Gurjar et al., 2022). Khan and colleagues emphasized that bitter gourd exhibits significant resistance to red pumpkin beetles. Similarly, the findings of Ahmad et al. (2020) and Saljoqi and Khan (2007) revealed that beetle infestation was highest on squash, with cucumber ranking second.

In this study, a higher number of beetles were observed on sponge gourd, followed by cucumber. The slight variation in results may be attributed to the presence of different species in various locations worldwide. In this study, the species under investigation was *Raphidopalpa foveicollis* (Lucas). These findings align closely with those of Mondal et al. (2020), who reported that the beetles completely disappeared in July. Mondal et al. also identified *R. foveicollis* as a significant pest of cucurbit crops.

The analysis of current study indicated that sponge gourd experienced the highest infestation, followed by cucumber, squash, and pumpkin, respectively. In contrast, the lowest infestation of red pumpkin beetles was recorded on bottle gourd. Studies by Regmi and Paudel (2020) and Khan et al. (2015) suggest that muskmelon exhibits a lower infestation rate of red pumpkin beetles. Although muskmelon was not included in this study, its proximate composition is similar to that of bottle gourd. Therefore, the low infestation rate observed for bottle gourd in this study is consistent with these findings.

Fluctuations in the monthly population of red pumpkin beetles on their preferred cucurbit hosts may be attributed to temperature changes during March, April, May, and June. Ahmad et al. (2020) reported that the beetle population peaked in April and May. Additionally, Regmi and Paudel (2020) observed that red pumpkin beetles remained active from March to October, with their peak activity occurring between April and June.

Fruit flies are significant pests in Pakistan, causing substantial losses to fruit and vegetable production at the farm level (Abbas et al., 2021), as well as affecting fleshy vegetables globally (Mondal et al., 2020). In this study, the maximum damage caused by fruit flies was observed on bitter gourd, followed by squash and sponge gourd, while the lowest damage was recorded on pumpkin and cucumber. These findings align with those of Kubar et al. (2021), who reported that the highest percentage of feeding occurred on bitter gourd in a study focusing on three species of *Bactrocera*: *B. zonata*, *B. longistylus*, and *B. cucurbitae*.

This study, however, focused exclusively on *B. cucurbitae*, and the results revealed that the maximum level of damage was observed on bitter gourd. These findings are consistent with those of Gaddanakeri and Rolania (2020) and Manoj et al. (2017), who stated that sponge gourd, bitter gourd, muskmelon, and snap melon were the most preferred and favored hosts of *B. cucurbitae*. They also reported that *B. cucurbitae* infested up to 95% of bitter gourd, followed by 60-87% infestation in pumpkin, with the lowest infestation observed in cucumber.

In this study, fruit flies were most active from late April to June. These findings are consistent with those of Barma et al. (2013), who reported that *B. cucurbitae* damage occurred from the last week of April to the second week of June during 2008 and 2009. They also indicated that infestation levels varied depending on fruit availability, growth stages, weather factors, and other environmental conditions.

In the current study, cucumber experienced less damage compared to sponge gourd, squash, and bitter gourd. These results align with the findings of Pilania et al. (2021) and Surender et al. (2016), which highlight variations in the nutritional composition of different vegetables. Such differences are important to consider when making dietary choices.

Chy et al. (2011) reported that bitter gourd contains 0.4% fat, 1.09% protein, and moisture content ranging from 91.6% to 92.92%. These results are consistent with those obtained in the current study. Similarly, Chunduri (2013) found that bitter gourd had a moisture content of 89.011%, a protein content of 1.17%, and a fiber content of 1.34%, which also aligns with the findings of this study.

Furthermore, Fedha et al. (2010) emphasized the importance of low moisture levels in pumpkin seeds, which facilitate long-term preservation. They also noted that Kenyan pumpkin species are particularly rich in proteins, making them an excellent dietary choice for children.

Overall, the findings of this study provide valuable insights into the nutritional composition of bitter gourd and pumpkin seeds. The similarities between the results of this study and previous research validate the accuracy and reliability of the data. This understanding of the biochemical properties of Cucurbitaceae species enhances our knowledge of host defense physiology, which can be leveraged in integrated pest management strategies.

CONCLUSION

The research findings indicated that *Aulacophora foveicollis* exhibited a strong preference for sponge gourd as its most favored host, while bottle gourd was the least preferred. Similarly, bitter gourd was identified as the preferred host for the fruit fly (*Bactrocera cucurbitae*), with pumpkin being the least chosen. Correlation analysis revealed that *A. foveicollis* showed a positive correlation with moisture, fat, and protein content, while demonstrating a negative correlation with fiber and ash content. Likewise, *B. cucurbitae* exhibited a positive correlation with moisture and protein content, but a negative correlation with fat, fiber, and ash content.

It is recommended to cultivate bottle gourd in areas where fruit fly infestation is prevalent. Conversely, in regions with high populations of *A. foveicollis*, bitter gourd should be grown due to its high crude fiber content.

AUTHORS' CONTRIBUTIONS

AL, TK, AA and MK conducted experiments and collected data; AL, AR and SI conceived the study plan; AR, SI, MSR and IH supervised the study; AL, MK and FIN wrote the initial manuscript; AL, TK and MK collected data; AL, AR and SI designed the study, analyzed data and finalized the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abbas, M., Hussain, D., Saleem, M., Ghaffar, A., Abbas, S., Hussain, N., Ghaffar, A., 2021. Integrated pest management of guava, citrus and mango fruit flies at three districts of Punjab. Pakistan Journal of Zoology 53(3), 995.

Abd El-Karim, H.S., Rizk, M.A., Mohamed, I.A., 2024. Biochemical and morphological characteristics of leaves and their relation with infestation of selective piercing-sucking pests on cucumber (*Cucumis sativus*), okra (*Abelmoschus esculentus*) and eggplant (*Solanum melongena*). International Journal of Agriculture and Biology 31(5) 383-392.

AACC., 2004. Approved methods of the American Association of Cereal Chemists, 10th Ed. Method 26-10A, 26-95, 30-10, 39-11, 44-11, 54-21, and 56-81B. The Association: St. Paul, MN.

AOAC., 1990. Official methods of analysis, Association of Official Analytical Chemists, Washington, D.C., USA. 15th Edition, pp. 807-928.

Ahmad, M.H., Afzal, M., Ullah, M.I., Mehmood, N., Ali, S., Arshad, M., Raza. A.B.M., 2020. Feeding preferences of red pumpkin beetle, *Aulacophora foveicollis* Lucas (Chrysomelidae: Coleoptera) on different cucurbit cultivars. Egyptian Academic Journal of Biological Sciences 13(02), 295-303.

Ajuru, M., Nmom. F., 2017. A review on the economic uses of species of Cucurbitaceae and their sustainability in Nigeria. Advanced Journal Of Plant Biotechnology 2(01), 17-24.

Akdura, N., Culal-Kilic. H., 2022. Molecular characterizations of cucumber mosaic virus and tomato mosaic virus isolates in Hakkari Turkey. Fresenius Environmental Bulletin 31(08), 8993-9004.

Barma, P., Jha. S., Banerjee. S., 2013. Prediction of population development of melon fruit fly (Bactrocera cucurbitae Coq.) on pointed gourd (*Trichosanthes dioica* Roxb.). African Journal of Agricultural Research 8(38), 4740-4747.

Baloglu, M.C., 2018. Genomics of cucurbits. In Rout GR, Peter KV (eds) genetic engineering of horticultural crops. Academic Press, pp. 413-432.

CABI., 2020. Invasive species compendium, datasheet report for *Bactrocera cucurbitae* (melon fly). https://www.cabi.org/isc/datasheetreport/17683

Chunduri, J.R., 2013. Antioxidant and nutritional analysis of edible cucurbitaceae vegetables of India. International Journal of Biology 2(08), 1124-1129.

Chy, F.K., Sarkar, S.K., Islam, M.K., Nurul. A., 2011. Nutrient and phytochemical analysis of four varieties of bitter gourd (*Momordica charantia*) grown in Chittagong hill tracts, Bangladesh. Asian Journal of Agricultural Research 5(03), 186-193.

Dhillon, N.P., Sanguansil, S., Singh, S.P., Masud, M.A.T., Kumar, P., Bharathi, L.K., McCreight. J.D., 2017. Gourds: bitter, bottle, wax, snake, sponge and ridge. Genetics and Genomics of Cucurbitaceae 155-172.

Fahad, S., Saud, S., Akhter, A., Bajwa, A.A., Hassan, S., Battaglia, M., Irshad. I., 2021. Bio-based integrated pest management in rice: An agro-ecosystems friendly approach for agricultural sustainability. Journal of the Saudi Society of Agricultural Sciences 20(02), 94-102.

Fedha, M.S., Mwasaru, M.A., Njoroge, C.K., Ojijo, N.O., Ouma. G.O., 2010. Effect of drying on selected proximate composition of fresh and processed fruits and seeds of two pumpkin species. Agriculture and Biology Journal of North America 1(06), 1299-1302.

Gaddanakeri, S., Rolania, K., 2020. Biology and morphometrics of melon fruit fly, *Bactrocera cucurbitae* Coquillett on bitter gourd (*Momordica charantia L*). Journal of Entomological and Zoological Society 8(05), 994-998.

Gurjar, M.K., Jat, B.L., Choudhary, P., Nayak. R.K., 2022. Screening of bottle gourd genotypes/varieties for resistance against red pumpkin beetle *Raphidopalpa foveicollis* (Lucas) in semi-arid region of Rajasthan. Indian Journal of Ecology 49(05), 1773-1781.

Grumet, R., McCreight, J.D., McGregor, C., Weng, Y., Mazourek, M., Reitsma, K., Fei. Z., 2021. Genetic resources and vulnerabilities of major cucurbit crops. Genes 12(08), 1222.

Hafsi, A., Abbes, K., Harbi, A., Ben-Othmen, S., Limem, E., Elimem, E., Ksantini, M., Chermiti, B., 2015. The pumpkin fly *Dacus frontalis* (Diptera: Tephritidae): A new pest of cucurbits in Tunisia. Bulletin 45(02), 209-213.

He, S., 2023. Complex ecological, physiological, and genetic analysis of the response of Cucurbita genus representatives to salt stress. Review of the official opponent for PhD thesis of He Songtao by Pavlo Pyasrenko 10(101), 1-5.

Hendrichs, J., Vreysen, M.J.B., Enkerlin, W.R., Cayol. J.P., 2021. Strategic options in using sterile insects for area-wide integrated pest management. In Sterile insect technique CRC Press (841-884).

Haldhar, S.M., Berwal, M.K., Samadia, D.K., Kumar, R., Gora, J.S., Choudhary, S., 2018. Biochemical basis of plant-insect interaction in arid horticulture crops: a scientific review. Journal of Agriculture and Ecology 6, 1-16.

Haldhar, S.M., Kumar, R., Corrado, G., Berwal, M.K., Gora, J.S., Thaochan, N., Basile, B., 2022. A field screening of a pomegranate (*Punica granatum*) ex-situ germplasm collection for resistance against the false spider mite (*Tenuipalpus punicae*). Agriculture 12(10), 1686.

Haldhar, S.M., Bhargava, R., Choudhary, B.R., Pal, G., Kumar, S., 2013. Allelochemical resistance traits of muskmelon (*Cucumis melo*) against the fruit fly (*Bactrocera cucurbitae*) in a hot arid region of India. Phytoparasitica 41, 473-481.

Karban, R., 2011. The ecology and evolution of induced resistance against herbivores. Functional Ecology 25, 339-347.

Kubar, M.I., Khoso, F.N., Khatri, I., Khuhro, N.H., Gilal. A.A., 2021. Effect of different management strategies on melon fruit fly, *Bactrocera cucurbitae* (Coquillett), infestation in cucurbit vegetables. Sarhad Journal of Agriculture 37, 915-920.

Kaur, R., Gupta A.K., Taggar, G.K., 2015. Induced resistance by oxidative shifts in pigeonpea (*Cajanus cajan* L.) following *Helicoverpa armigera* (Hübner) herbivory. Pest Management Science 71, 770-782.

Khan, L., Shah, M., Usman, A., 2015. Host preference of red pumpkin beetle (*Aulacophora faveicollis*) Lucas (Chrysomelidae: Coleoptera) among different Cucurbits. Journal of Entomology and Zoology Studies 3(2), 100-104.

Kariyat, R.R, Balogh, C.M., Moraski, R.P., De Moraes, C.M., Mescher, M.C., Stephenson. A.G., 2013. Constitutive and herbivore-induced structural defenses are compromised by inbreeding in *Solanum carolinense* (Solanaceae). Journal of Botany 100, 1014-1021.

Li, Y., Fan, H., Si, Y., Guo, Y., Wu, Z., Du C., 2023. The phloem lectin PP2-A1 enhances aphid resistance by affecting aphid behavior and maintaining ROS homeostasis in cucumber plants. International Journal of Biological Macromolecules 229, 432-442.

Manoj, A., Sridharan, M., Mohan, S., Awasthi, Nikita, S., 2017. Varying infestation of fruit fly, *Bactrocera Cucurbitae* (Coquillett) in different cucurbit crops. Journal of Entomological and Zoological Society 5 (03), 1419-1421.

Mondal, B., Mondal, C.K., Mondal, P., Mondal, B., Mondal, C.K., Mondal, P., 2020. Insect pests and non-insect pests of cucurbits. Stresses of cucurbits: status and management*.* 47-113.

Pilania, S., Yadav, S.S., Rolania, K., Kumar, R., Indora, J., Kumar, A., 2021. Assessment of avoidable yield losses in bitter gourd due to melon fruit fly, *Bactrocera cucurbitae* (Coquillett). Journal of Entomology and Zoology Studies 9(02), 396-398.

Piñero, J.C., Quinn, J., Byers, P., Miller, P., Baker, T., Trinklein, D., 2015. Knowledge and use of integrated pest management by underserved producers in Missouri and the role of extension. Journal of Extension Education 53(3), 17.

Rai, A.B., Halder, J., Kodandaram, M.H., 2014. Emerging insect pest problems in vegetable crops and their management in India: An appraisal. Pest Management in Horticultural Ecosystems 20(02), 113-122.

Regmi, S., Paudel, M., 2020. A review on host preference, damage severity and integrated pest management of red pumpkin beetle. Environmental Contamination and Toxicology 3, 16-20.

Saljoqi, A.U.R., Khan, S., 2007. Relative abundance of the red pumpkin beetle, *Aulacophora foveicollis*, on different cucurbitaceous vegetables. Sarhad Journal of Agriculture 23(01), 109-114.

Sajeevan, R.S., Abdelmeguid, I., Saripella G.V., 2023. Comprehensive transcriptome analysis of different potato cultivars provides insight into early blight disease caused by *Alternaria solani*. BMC Plant Biology 23, 130-135.

Siddiqui, J.A., Fan, R., Naz, H., Bamisile, B.S., Hafeez, M., Ghani, M.I., Chen, X., 2023. Insights into insecticide-resistance mechanisms in invasive species: Challenges and control strategies. Frontier Physiology 13, 1112278.

Sapkota, S.N., Bollinger, L., Klinger, Y., Tapponnier, P., Gaudemer, Y., Tiwari, D., 2013. Primary surface ruptures of the great Himalayan earthquakes in 1934 and 1255. Nature Geoscience 6(1), 71-76.

Steel, R.G.D., Torrie, J.H., 1980. Principal and procedure of statistics; A biological approach. 2nd Ed. Mcgraw Hill book co. New York. p. 481.

Surender. K.S., Punam, A., Kumar, R., 2016. Management of fruit fly (*Bactrocera* spp.) in cucumber (*Cucumis sativus* L.) grown organically. Journal of Biopesticide 9 (01), 73-79.

Subedi, K., Regmi, R., Thapa, R.B., Tiwari S., 2021. Evaluation of net house and mulching effect on Cucurbit fruit fly (*Bactrocera cucurbitae* Coquillett) on cucumber (*Cucumis sativus* L.). Journal of Agriculture Research 3, 100103.

War, A.R., Paulraj, M.G., Ahmad, T., Buhroo, A.A., Hussain, B., Ignacimuthu, S., Sharma, H.C., 2012. Mechanisms of plant defense against insect herbivores. Plant signaling & Behavior 7(10), 1306-1320.

Xue, W., Haynes, K.G., Clarke, C.R., Qu, X., 2022. Genetic dissection of early blight resistance in tetraploid potato. Frontiers in Plant Science 13, 851538.