Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(3): 960-964

Received: 25-03-2020
Accepted: 27-04-2020

Dr. Garima Gupta
Associate Professor, UIAS Chandigarh University, Gharuan, Punjab, India

Shirin Sharma
Associate Professor, UIAS Chandigarh University, Gharuan, Punjab, India

Dr. Neelima Kumar
Professor, Department of Zoology, Panjab University, Chandigarh, India

Corresponding Author:
Dr. Garima Gupta
Associate Professor, UIAS Chandigarh University, Gharuan, Punjab, India

Carica papaya aqueous leaf extracts as potential botanical insecticide against rose aphids (*Macrosiphum rosaeformis* D.)

Dr. Garima Gupta, Shirin Sharma and Dr. Neelima Kumar

Abstract
Current study elucidates the feasibility of the use of *Carica papaya* plant leaf aqueous (AQ) extracts for the control of economically serious pest *Macrosiphum rosaeformis*, rose aphids. Lethal concentrations obtained were 3.39 and 0.06 g/ml at 24, 48 and 72 hr treatment. The residual bioassays indicated that the papaya leaf extract was effective till 11DAT at 24hr and had significantly higher mortality than control. Further, at 48 hr it was effective till 13DAT and had significantly higher mortality than control. Spectrophotometric estimation of flavonoids in papaya revealed higher concentration in the aqueous leaf extracts of papaya (61.24 g/ml) than in the Soxhlet MeOH extract (20.85 g/ml). The observations will be helpful in developing an IPM module with possible use of these plant extracts in ornamental greenhouse crops. This study for the first time reported the toxic effect of papaya leaf aqueous extract on rose aphids.

Keywords: *Carica papaya*, botanical insecticide, *Macrosiphum rosaeformis*, flavonoid, bioassay

Introduction
Carica papaya belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases [1]. The whole plant has its own medicinal value. Papaya nutritionally is an excellent source of three powerful antioxidant vitamin C, vitamin A and vitamin E; the minerals, magnesium and potassium; rich in B complex vitamins, pyridoxine (vitamin B6) and folic acid [2, 3]. *Carica papaya* is a neutraceutical plant having a wide range of pharmacological activities. Papaya can eliminate intestinal parasites, cure for piles and typhoid, anti-helminthic and anti-amoebic properties [4] and even useful in treatment of dengue fever [5]. The antihyperglycemic effect of unripe mature fruits and seeds of *C. papaya* have also been reported [6, 7]. The aqueous extract of *C. papaya* leaves were investigated for evaluation of wound healing potential in rats [8]. Besides all other biological activities *C. papaya* also been exhibited insecticidal [9, 10], fungicidal and microbicidal [11, 12, 13] effects.
Important biologically active compounds have been identified in papaya leaves [14, 15], where they function in metabolism, defence, signalling and protection from excess light, among others [16, 17]. Seven flavonoids were isolated from MeOH extract of *C. papaya* leaves, including quercetin and kaempferol [18]. Flavonoids (Bioflavonoids) are secondary metabolites of plants present in all photosynthesizing cells and if present in leaves are believed to function to protect and promote physiological survival of plant from for instance UV radiations and fungal pathogens [19]. A role is played by Flavonoids in protecting plants against herbivores and plant feeding insects [20]. Their presence makes plants unpalatable, decrease digestibility, act as toxins or even reduce their nutritive value. Feeding tests have shown a number of insects sensitive to flavonoid compounds [21].
Naturally occurring flavonoids obtained from wheat extracts have been examined for feeding deterrency of wheat extracts towards two aphid species, *Schizaphis graminum* and *Myzus persicae* [22]. The cultivated lines of cowpea (*Vigna unguiculata* L.) possess quercetin, kaempferol, and isorhamnetin, as major flavonoids which were responsible for resistance against aphids [23]. Three flavonoids viz., quercetin dehydrate and rutin hydrate and naringine proved to cause mortality of woolly apple aphid, *Eriosoma lanigerum* at very low concentration i.e.1000ppm [24]. Flavonoids genistein and luteolin when incorporated with artificial diets of pea aphid, *Acyrthosiphon pisum* detrimental effects on the feeding behavior was observed [25]. The studies suggested that plant parts have immense potential as a source of

biologically active agents that may have a promising role in the management of various insect pests.

In view of the need to develop a biological control program for garden ornamentals with the aim to reduce pollution load on the environment at the same time maintaining the market value of ornamentals, the present study was undertaken. The aim is to evaluate the efficacy of aqueous leaf extracts of papaya (*Carica papaya* L.) against the rose aphis (*Macrosiphum roseiformis* D.). In addition to this, field bioassay was performed to check the residual effect of leaf extract. Soxhlet extraction (methyl alcohol) and estimation of flavonoids was done to compare the flavonoid concentrations of aqueous extracts and alcoholic extracts.

Materials and Methods

Aqueous extract (papaya leaves) have been bioassayed (laboratory and field) against aphids, *Macrosiphum rosaeformis* D. in the present study. In addition, Soxhlet extraction (methyl alcohol) and spectrophotometric estimation of flavonoids was done to compare the flavonoid concentrations of aqueous extracts and alcoholic extracts. The study material and experimental procedures are detailed below:

Plant and Insect material used for experimentation

The variety Giant rose, *Rosa* sp. was used in present study for bioassays. These were obtained from plant nursery, which were maintained in glass house at Department of Zoology, Panjab University, Chandigarh, India. Leaves of cultivated papaya (*Carica papaya* L.) were collected from locations in and around Chandigarh. The samples of these plants were sent to Department of Botany, Panjab University, Chandigarh for identification and catalogue number 21121 was obtained. Natural populations of rose aphids (*Macrosiphum roseiformis* D.) were collected from Rose garden, Panjab University, Chandigarh. Aphids were reared on rose plants for few generations and were used for experimentation later.

Procedure for aqueous extract preparation

The leaves of papaya (*Carica papaya*) were obtained and cleaned with water and rectified alcohol. These leaves were then dried in oven (dehydrator) at 40-50°C till the leaves were completely dried for grinding. Powdered plant material was then used for the preparation of aqueous extract. 200 ml of distilled water was added to 50g of leaf powder which was mixed thoroughly in a mixer grinder for 5min. and later was squeezed and sieved properly with muslin cloth. This concentration (X1 = 0.25g/ml) was considered as stock solution and was the highest concentration used in bioassay experiments.

Efficacy studies

Laboratory bioassay

Extract Preparation: For aqueous plant extract the stock solution or highest concentration was considered (X1= 0.25 g/ml). Further five serial dilutions were made to obtain as X2= 0.125 g/ml, X3=0.0625 g/ml, X4=0.03125 g/ml, X5=0.015625 g/ml and X6=0.0078125 g/ml. Extracts were refrigerated and prepared fresh after seven days.

Experiment Plan: Compound rose leaflets were collected and washed thoroughly with water. For each treatment, three replications and six concentrations of each were taken along with one untreated control. Treated leaves were kept in petridish (90 x 15mm). For control untreated leaflets kept which were washed only with water. In every petridish ten third instar aphids were released. For the entire experiment one biological replicate was also conducted at different date. For observations survival number were observed at 24, 48 and 72 hr after treatment. Median lethal Concentration (LC_{50}) values were calculated by computer programme POLO-PC (Copyright LeOra Software 1987).

Field bioassay

The highest concentration of extract and untreated control, were taken for residual experiments. 20 rose plants were grown in floral foam (10cm x 6.5cm x 7cm) and taken for field bioassay. For each treatment four replicates were taken and placed randomly in different rows. On first day of experiment plants were treated with required concentrations of extract. After 24 hours of treatment two samples of leaves were randomly collected from each plant and were placed in different petridishes, 10 insects (aphids) were released and number of survived insects was observed after 24 and 48 hours. Similar steps were repeated on 3rd day, 5th day, 7th day, 9th day, 11th and 13th day of experiment. Data were analyzed by all replicates combined with ANOVA and means were compared using Students 't' test [26].

Soxhlet extraction of flavonoids

Papaya plant materials were dried and grounded. 20g of the powdered sample of both the plants was taken wrapped with filter paper and placed in the extraction chamber of the Soxhlet apparatus. Methanol 80% was taken as solvent and was poured in distillation flask of Soxhlet apparatus. The temperature of heat source was set at 50-60°C. The Soxhlet was run for 20 hours and then the extract was collected. The alcoholic extract was taken in a conical flask, sealed and refrigerated for further experiments.

Quantification of flavonoids by Spectrophotometric analysis

To evaluate the concentration of flavonoids in the extracts, quantification was done following methods discussed by Fajrin [27] and Hoque [28]. For standard sample quercetin five serial dilutions was prepared: 1mg/ml, 0.5, 0.25, 0.125, 0.062 mg/ml. For the different test extracts: 500 µl of sample was mixed with 2 ml of distilled water. Then by following above mentioned steps addition of $NaNO_3$ (25%), $AlCl_3$ (10%), NaOH (4%) and distilled water the volume of 5 ml was obtained for all samples. The O.D. was taken at 510 nm with the help of Spectrophotometer

Calculations

Flavonoid concentration was calculated using following formula:

$$\text{Flavo. conc. of sample } (\mu g/ml) = \frac{\text{OD of sample} - \text{OD of blank}}{\text{OD of standard} - \text{OD of blank}} \times \text{Conc. of standard}$$

Results and Discussion

Laboratory bioassays: Papaya leaf extract (AQ) showed significantly higher values than control for percentage mortality (mortality range- 20%-70%) of aphids at all concentrations from X1 (0.25 g/ml) to X6 (0.0078125 g/ml) tested at 72 hr post treatment. However, at 24 hr, the values were not significantly higher than control and mortality range was 10%- 30%. At 48 hr mortality range was 10%- 50%, which was significantly higher than control only at the

concentrations i.e. X1, X2, X3 and X4 (Fig. 1). At 24 hr LC_{50} (3.39) was 13 times higher than the highest concentration tested (X1). Besides, at 48 hr the LC_{50} value (0.25) was the same as the highest concentration taken (X1) and at 72 hr the LC_{50} value (0.06) was the lowest and was equal to the X3 concentration. Results suggested that this extract was toxic to aphids only at higher concentrations (Table 1).

Field bioassays: The residual bioassays with *papaya* leaves indicated that the papaya leaf extract AQ was effective till 11DAT at 24hr and had significantly higher mortality than control. Further, at 48 hr it was effective till 13DAT and had significantly higher mortality than control (Fig. 2).

Evaluation of flavonoids: The flavonoids evaluation was done using the spectrophotometer and taking quercetin as standard as mentioned in material and methods.

Figure 3 shows the flavonoid estimation of papaya. Papaya SE (Soxhlet MeOH extract) showed 20.85 g/ml concentration of flavonoids while papaya AQ (aqueous extract) was estimated to have 61.24 g/ml concentration of flavonoids. These results clearly indicated that the concentration of flavonoids recorded obviously higher in the aqueous leaf extracts of papaya than in the Soxhlet MeOH extract.

C. papaya has been reported to show lethal and antigrowth effects against three lepidopteran pests viz., *Samia ricini* and *Mamestra brassicae* and *Spodoptera litura* [17]. *C. papayam*, *Murraya paniculata* and *Cleistanthus collinus* seed, fruit and leave extracts were evaluated for their larvicidal properties against *Culex quinquefasciatus.* In accordance to our study during which highest mortality for all concentrations tested was noted at 72 hr, this study also suggested higher mortality rates at 72h observations than that of 24 and 48 h bioassay [29]. Efficacy of leaf powder of *C. papaya* was tested against *Sitophilus zeamais* which proved toxic and repellent effects of the pest in stored maize grain [10]. Larvicidal and pupicidal activity of *C. papaya* was investigated against *Aedes aegypti*. The leaf powder was extracted with organic solvent methanol using a Soxhlet apparatus. The plant extract showed larvicidal and pupicidal effects after 24 h of exposure; however, the highest larval and pupal mortality was against the first- to fourth-instar larvae and pupae [30]. During present studies, however, addition of either *Pseudomonas* or *Bacillus* to papaya leaf extract was seen to reduce the LC_{50} dose perhaps because the aqueous extract used in the present study had higher flavonoids content than methanol extract obtained after Soxhlet extraction as observed after Spectrophotometer quantification analysis.

In corroboration of present results, the leaf extract of *C. papaya* was to shown to have insecticidal activity against *Aphis gossypii*, *Earias vittella*, *Phytoecia puncticollis* and *Bemisia tabaci*. The spraying frequency of the extract was once in a week. It was observed that papaya leaf extract at lower concentration of treatments failed to control the pest infestation while at higher concentration it effectively reduced pest infestation [31]. Papaya leaf extract reported insecticidal activity against mustard aphid which is similar to observations received in the present study [32]. One ml of leaf extract concentration was sprayed on mustard aphid (*Lipaphis erysimi*) population on leaf in the bioassay chamber. Papaya leaf extract showed insecticidal activity to aphid with LC_{50} value 87.0 ppm.

Similarly in our other study with other botanical products, field bioassays have been conducted with wild pomegranate *daru* peel and seeds to check percentage mortality of aphids at different days after treatment (DAT) [33] and a significant increase in mortality was recorded at both 24 and 48 hr of exposure on treated leaves than control up to 7 DAT. In a different study, field bioassays with lemon peel aqueous extract as six different concentrations revealed that all were equally effective till 9DAT against aphids and toxicity was directly proportional respective to increase in concentration [34]. Crude aqueous seed extracts of *Annona squamosa* exhibited efficacy compared to pyrethrum against larvae of diamond back moth, *Plutella xylostella* L [35]. The effectiveness of two insecticidal formulations of botanical origin having mixtures of *Piper retrofractum* and *A. squamosa* extracts and *Aglaia odorata* and *A. squamosa* extracts was assessed [36]. Mixtures at 0.1% concentration found to be more effective in controlling populations of *Crocidolomia pavonana* and *P. xylostella* in comparison to synthetic pyrethroid insecticide deltamethrin at 0.04% and the microbial insecticide *Bacillus thuringiensis* at 0.15% in the field. A moderate toxicity of six different botanical extracts viz., *Citrus sinensis*, *Momordica dioica*, *Allium vineale*, *Capsicum frutescens*, and *Nicotiana tabacum*on wheat aphid [37].

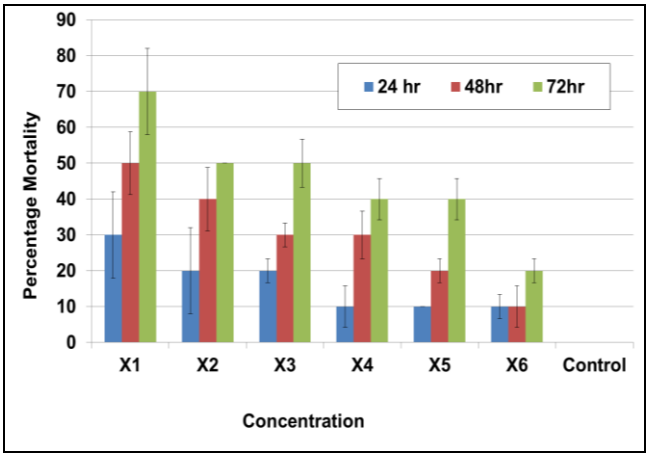

Fig 1: Percentage mortality of aphids with papaya leaf extract for laboratory bioassays (p<0.05).

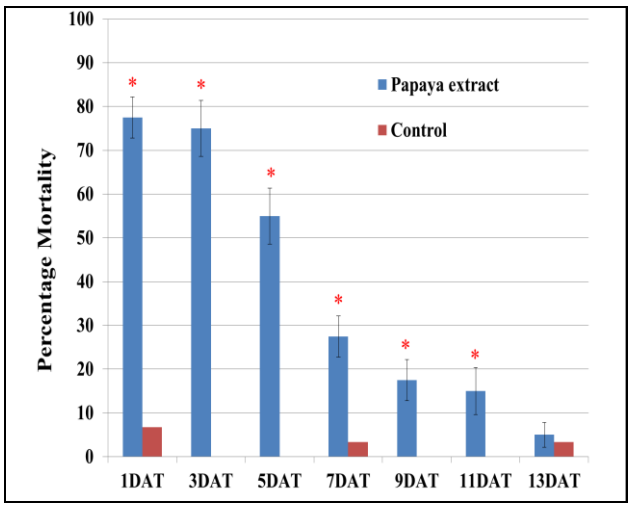

Fig 2: The percentage mortality of aphids at different days after treatment (DAT) for field bioassays (p<0.05).

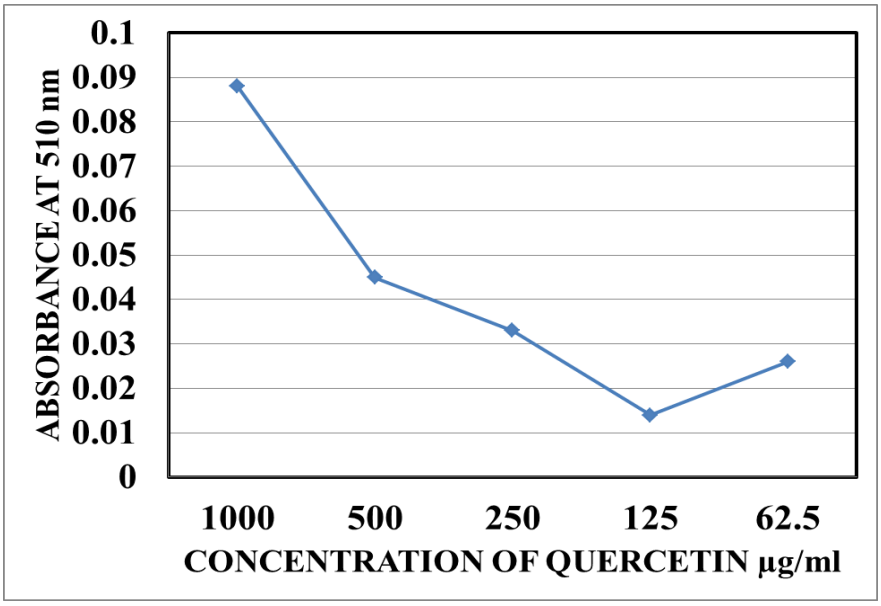

Fig 3: Standard curve for flavonoid estimation

Table 1: LC_{50} (g/ml) of papaya leaf extract and quercetin against aphids.

Treatment	24hr			48hr			72hr		
	LC_{50} (g/ml)	Slope	χ^2	LC_{50}	Slope	χ^2	LC_{50}	Slope	χ^2
Papaya	3.398	0.5	0.3	0.252	0.7	0.2	0.066	0.7	0.7
Quercetin	0.068	0.6	0.4	0.043	0.6	0.1	0.008	0.7	0.4

Conclusion

Present results suggested that higher flavonoids content was obtained in aqueous extract as compared to methanol extract from Soxhlet extraction. On the basis of results obtained in experimental assays it can be concluded that papaya leaf extract were highly toxic to aphids as its LC_{50} value at 72 hr was equal to X3 concentration. Field bioassays also proved the efficacy of the extract against these pests up to more than a week period under green house conditions. On the basis of these results, papaya leaf extracts proved safe biological pesticide and showed moderate to high toxicity towards the sucking pests of rose. These could be recommended as a possible tool to incorporate with other pest management practices in greenhouse IPM. The leaves of these plants can be obtained in a large quantity as they are very commonly grown in India.

Acknowledgement

Financial support provided by DST, Ministry of Science and Technology, India, is greatly acknowledged. Facilities and workspace were provided by Department of Zoology, Panjab University, Chandigarh, which is gratefully acknowledged.

References

1. Alabi OA, Haruna MT, Anokwuru CP, Jegede T, Abia H, Okegbe VU and Babatunde EE. Comparative studies on antimicrobial properties of extracts of fresh and dried leaves of *Carica papaya* (L.) on clinical bacterial and fungal isolates. Adv. Appl. Sci. Res. 2012; 3: 3107-3114.
2. Wall MM. Ascorbic acid, vitamin A, & mineral composition of banana & papaya cultivars grown in Hawaii. Journal of Food Composition and Analysis. 2006; 19 (5):434-445.
3. Yogiraj V, Goyal PK, Chauhan CS, Goyal A, and Vyas B. *Carica papaya* Linn: an overview. Int. j. herb. Med. 2014; 2(5): 1-08.
4. Aravind G, Debjit B, Duraivel S and Harish G Traditional and medicinal uses of *Carica papaya*. J Med. Plants Stud. 2013; 1: 7-15.
5. Ahmad N, Fazal H, Ayaz M, Abbasi B H, Mohammad I and Fazal L. Dengue fever treatment with *Carica papaya* leaves extracts. Asian Pac. J Trop. Biomed. 2011; 1(4): 330-333.
6. Olagunja JA, Ogunlana CO and Gbile Z. Preliminary studies on the hypoglycemic activity of ethanolic extract of unripe, mature fruits of *Carica papaya*. Nig. J Biochem. Mol. Biol. 1995; 10: 2-3.
7. Adeneye AA and Olagunju JA. The aqueous seed extract of *Carica papaya* Linn. prevents carbon tetrachloride induced hepato-toxicity in rats. Int. J appl. res. nat. prod. 2009; 2: 19-32.
8. Mahmood AA, Sidik K and Salmah I. Wound healing activity of *Carica papaya* L. aqueous leaf extract in rats. J Mol. Med. Adv. Sci. 2005; 1: 398-401.
9. Perez-Gutiérrez S, Zavala-Sánchez MA, González-Chávez MM, Cárdenas-Ortega NC and Ramos-López MA. Bioactivity of *Carica papaya* (Caricaceae) against *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Molecules. 2011; 16: 7502-7509.
10. Muzemu S, Chitamba, J and Mutetwa1 B. Evaluation of *Eucalyptus tereticornis*, *Tagetes minuta* and *Carica papaya* as stored maize grain protectants against *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae). Dept. Agri. For. Fisheries. 2013; 2: 196-201.
11. Omojasola PF and Awe S. The antibacterial activity of the leaf extract of *Anacardium occidentale* and *Gossypium hirsutum* against some selected microorganisms. Plant Sci. 2004; 2: 447-445.
12. Udoh P, Essien I and Udoh F. Effect of *Carica papaya* (paw paw) seeds extract on the morphology of pituitary-gonadal axis of male Wistar rats. Phytother. Res. 2005;

19: 1065-1068.
13. Romasi EF, Karina J and Parhusip, AJN. Antibacterial activity of papaya leaf extracts against pathogenic bacteria. Makar. Tehnologi. 2011; 15: 173-177.
14. Canini A, Alesiani D, D'Arcangelo G and Tagliatesta P. Gas chromatography-mass spectrometry analysis of phenolic compounds from *Carica papaya* L. leaf. J Food Compos. Anal. 2007; 20: 584-590.
15. Zunjar V, Mammen D, Trivedi BM and Daniel M. Pharmacognostic, physicochemical and phytochemical studies on *Carica papaya* Linn. leaves. J Pharm. 2011; 3:5-8.
16. Moussaoui EA, Nijs M, Paul C, Wintjens R, Vincentelli J, Azarkan M and Looze Y. Revisiting the enzymes stored in the laticifers of *Carica papaya* in the context of their possible participation in the plant defence mechanism. Cell Mol. Life Sci. 2001; 58: 556-570.
17. Konno K, Hirayama C, Nakamura M, Tateishi K, Tamura Y, Hattori M and Kohno K. Papain protects papaya trees from herbivorous insects: role of cysteine proteases in latex. Plant J. 2004; 37: 370-378.
18. Nugroho A Heryani H, Choi JS and Park HJ. Identification and quantification of flavonoids in *Carica papaya* leaf and peroxynitrite-scavenging activity. Asian Pacific Journal of Tropical Biomedicine. 2017; 7(3): 208-213. DOI: 10.1016/j.apjtb.2016.12.009
19. Harborne JB and Baxter H. In: Handbook of Natural Flavonoids. John Wiley and sons, Chichester UK. 1838 p; 1999.
20. Harborne JB and Williams CA. Advances in flavonoid research since 1992. Phytochemistry. 2000; 55:481-504.
21. Treutter, D. Significance of flavonoids in plant resistance and enhancement of their biosynthesis. Plant Biol. 2005; 7: 581-591.
22. Dreyer DL and Jones KC. Feeding deterrency of flavonoids and related phenolics towards *Schizaphis graminum* and *Myzus persicae*: Aphid feeding deterrents in wheat Phytochemistry. 1981; 20(11):2489-93. https://doi.org/10.1016/0031-9422(81)83078-6
23. Lattanzio V, Arpaia S, Cardinali A, Di Venere D, Linsalata V. Role of endogenous flavonoids in resistance mechanism of Vigna to aphids. J Agric Food Chem. 2000; 48(11):5316-20.
24. Ateyyat M, Abu-Romman S, Abu Darwish M and Ghabeish I. Impact of Flavonoids against Woolly Apple Aphid, *Eriosoma lanigerum* (Hausmann) and Its Sole Parasitoid, *Aphelinus mali* (Hald.). Journal of Agricultural Science. 2012; 4:227-236. 10.5539/jas.v4n2p227.
25. Goławska S and Łukasik I. Antifeedant activity of luteolin and genistein against the pea aphid, *Acyrthosiphon pisum*. Journal of Pest Science. 2012; 85:443-450.
26. JMP SAS Institute. SAS for Windows, version 6.0.0. SAS Institute, Cary, NC. 2005.
27. Fajrin A and Tunjung WAS. The flavonoids contents in leaves and fruits of papaya (*Carica papaya* L.) var. california and var. gandul. KnE Life Sci. 2015; 2:154-158.
28. Hoque N, Faysal AA, Ahmed I, Chowdhury NS. Phytochemical screening and *in vitro* bioactives of the various extracts of *Carica papaya* leaves in Bangladesh. J Pharmacol. Pharmacother. 2015; 4: 42-46.
29. Rawani A, Haldar KM, Ghosh A and Chandra G. Larvicidal activities of three plants against filarial vector *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol. Res. 2009; 105:1411-1417.
30. Kovendan K, Murugan K, Kumar NA, Vincent S and Hwang JS. Bioefficacy of larvicdial and pupicidal properties of *Carica papaya* (Caricaceae) leaf extract and bacterial insecticide, spinosad, against chikungunya vector *Aedes aegypti* (Diptera: Culicidae). Parasitology Res. 2012; 110: 669-78.
31. Zobayer N and Hasan R. Effects of manually processed bio-pesticides on crop production and pest managements in okra (*Abelmoschus Esculentus* (L.) Moench). J Nat. Sci. Res. 2013; 3: 112-115.
32. Ahmed AU, Khanzada M and Shahzad S. Insecticide and papaya leaf extract toxicity to mustard aphid (*Lipaphis erysimi* Kal.). 2014; J Agri-food Appl. Sci. 2: 45-48.
33. Gupta G, Dharma K and Kumar NR. Insecticidal Effects of Aqueous Extracts of Wild Pomegranate Peel and Seed (*Punica granatum* L.) against *Macrosiphum rosaeformis* on Rose. Journal of Applied and Natural Science. 2017a; 9(3) 1397-1405.
34. Gupta G, Agarwal U, Kaur H, Kumar NR and Gupta P. Aphicidal effects of terpenoids present in *Citrus limon* on *Macrosiphum roseiformis* and two generalist insect predators. Journal of Asia Pacific Entomology. 2017b; 20(4):1087-109.
35. http://dx.doi.org/10.1016/j.aspen.2017.08.007
36. Leatemia JA and Isman MB. Efficacy of crude seed extracts of *Annona squamosa* against diamondback moth, *Plutella xylostella* L. in the greenhouse. Int. J Pest. Manag. 2004; 50:129-133.
37. Dadang EDF and Prijono D. Effectiveness of two botanical insecticides formulations to major cabbage insect pests on field application. J Int. Soc. Southeast Asian Agric. Sci. 2009; 15:42-51.
38. Iqbal MF, Kahloon MH, Nawaz MR and Javaid MI. Effectiveness of some botanical extracts on wheat aphids. J Anim. Plant Sci. 2011; 21:114-115.