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# ASSOCIATION OF TOTAL FLAVONOID CONTENT WITH RESISTANCE TO THE SAFFLOWER APHID, *Uroleucon compositae* (THEOBALD), IN DIVERSE SAFFLOWER (*Carthamus Tinctorius* L.) GENOTYPES

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**ABSTRACT**

Safflower (*Carthamus tinctorius* L.) is an economically important oilseed crop belonging to the family Asteraceae and is widely cultivated in semi-arid regions during the rabi season. The crop is valued for its high oil content as well as its rich repository of bioactive, medicinal, and aromatic compounds. Among the insect pests infesting safflower, the safflower aphid, *Uroleucon compositae* (Theobald), is considered the most destructive pest of safflower. The present study was conducted at the Instructional cum Research Farm and Laboratory of the Department of Entomology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, during the *Rabi* season of 2023-24. A total of 52 genotypes were evaluated under field conditions based on Aphid Infestation Index (A.I.I.) and aphid population per 5 cm central apical twig. The genotypes were classified into Resistant (R), Moderately Resistant (MR) and Susceptible (S) categories based on A.I.I. The total flavonoid content was estimated using the aluminium chloride method. Resistant genotypes accumulated significantly greater concentrations of total flavonoids than moderately resistant and susceptible genotypes. A significant negative correlation (r=-0.78174***) was observed between flavonoid content and aphid population indicating increased flavonoid content was associated with reduced aphid infestation. Regression analysis further confirmed the significant effect of flavonoid content on A.I.I. ($R^2$ = 0.7237) and aphid population ($R^2$ = 0.6111), while ANOVA and Tukey's HSD test showed significant differences among genotype categories. The findings suggest that flavonoids play an important role in imparting aphid resistance and may serve as reliable biochemical indicators for the identification and development of aphid-resistant safflower cultivars, thereby contributing to sustainable and eco-friendly pest management strategies.

***Keywords***: Aphid Infestation Index, safflower genotypes, correlation, regression, ANOVA, Tukey's HSD test, resistant cultivars.

## Introduction

*Carthamus tinctorius* L, commonly known as Safflower (Kusumbha, kusum, kardi), is a cool-season crop, specifically belonging to the Asteraceae family (Mani *et al*., 2020). In India, Safflower has traditionally been cultivated for its brightly coloured florets, which contain the commercially important pigment carthamin. This crop is also recognised as a significant source of aromatic and medicinal compounds (Ren *et al.*, 2025). That crop is valued for its high oil content. The safflower seed contains 24-36 per cent oil, which is devoid of flavour and colour. The oil possesses high nutritional value owing to its elevated proportion of unsaturated fatty acids. Safflower primarily yields three products: oil, meal, and birdseed. In addition, the crop is rich in vitamin A, iron, phosphorus, and calcium. Its oil is also utilized in the manufacture of paints, varnishes, coatings, and linoleum products. (Delshad *et al*., 2018).

India is among the leading safflower-producing countries and occupies a prominent position in global safflower cultivation contributing about 14.67 per cent of area and 9.02 per cent of production. In India, Safflower is cultivated over an area of <1 lakh hectares and produces 0.67 lakh tonnes with a productivity of 679 kg $ha^{-1}$. The Major safflower-growing regions in India include Telangana, Maharashtra, Andhra Pradesh, Karnataka, and Gujarat (Parameshnaik *et al*., 2023).

More than 36 insect species have been reported to infest safflower under Indian agro-ecological conditions, among them three insects identified as major pests: The *Uroleucon compositae* (Theobald) safflower aphid, the *Helicoverpa armigera* cotton bollworm, and the *Perigea capensis* leaf-eating caterpillar. The most damaging among these three pests is the safflower aphid, which causes a yield loss of 35-75 per cent during the duration of the infestation. Aphids are phloem-feeding insects that extract plant sap from tender tissues, thereby impairing plant growth and productivity (Wang *et al*., 2021). Heavy aphid infestations can induce chlorosis, leaf curling, stunted growth, and facilitate the transmission of plant viruses, which ultimately reduces yields. For increasing the safflower productivity, effective aphid management has to be followed (Jimenez *et al*., 2021, Simon *et al*., 2021). In order to boost yield, it is essential to decrease losses due to insect pests and balance the increasing global demand for oilseeds. The development and utilisation of resistance cultivars/genotypes constitute an important part of integrated and sustainable pest management as they reduce the dependence on chemical plant protection measures and help maintain pest populations below ETL's (Economic Threshold Levels) (Kloth *et al*., 2021).

In recent years, considerable research attention has been directed toward plant secondary metabolites involved in defense against herbivorous insects. (Divekar *et al.*, 2022). More than 50,000 secondary metabolites including terpenes, flavonoids and alkaloids have been identified in the plant kingdom. These compounds contribute significantly to constitutive and induced defense mechanisms against insect herbivores such as anti-feedant activity, repellency, inhibition of growth, reproduction and other physiological processes (Zhang *et al*., 2025, Ukorojie and Otayor, 2020). Flavonoids are among the major bioactive compounds in safflower and are known to significantly contribute to plant defense responses (Ren *et al.*, 2025).

Although there are several studies that reported the role of biochemical compounds in imparting resistance against insect pests, information on the association between flavonoid content and resistance to safflower aphid, *Uroleucon compositae* (Theobald), in safflower genotypes remains limited. Elucidating this relationship is essential for identifying potential biochemical markers that may facilitate the development of aphid-resistant safflower cultivars.

Therefore, the present study was undertaken to evaluate safflower genotypes for their resistance against *Uroleucon compositae* (Theobald) by assessing flavonoid content, Aphid Infestation Index and aphid population and to determine the relationship between these parameters.

## Materials and Methods

The present investigation was carried out at the Instructional cum Research Farm and Laboratory of the Department of Entomology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, C.G., during the *Rabi* season of 2023-24.

### Aphid Infestation Index

The aphid infestation index (A.I.I.) was determined by considering the grades of foliage drying. According to AII, germplasm accessions were classified into various categories (Table 1). The aphid infestation index (AII) was calculated using a formula recommended by entomologists involved in the All India Coordinated Research Project on safflower.

Aphid infestation index (A.I.I.) =

$$\frac{1Xa+2Xb+3Xc+4Xd+5Xe}{a+b+c+d+e}$$

Where,

a, b, c, d, e was the actual number of plants falling in each of the 5 corresponding foliage drying grades, *i.e.*, 1 to 5 scale. The mean of A.I.I. was worked out and the entries were classified as follows:

**Table 1:** Screening Techniques of safflower aphids based on foliage drying

| Sl. No. | Categories of genotypes | A.I.I |
|---|---|---|
| 1 | Highly Resistant (HR) | 0.0-1.0 |
| 2 | Resistant (R) | 1.1-2.0 |
| 3 | Moderately Resistant (MR) | 2.1-3.0 |
| 4 | Susceptible (S) | 3.1-4.0 |
| 5 | Highly Susceptible (HS) | >4.1 |

### Flavonoid content assessment

Total flavonoid content was estimated using the aluminium chloride method described by Zhishen *et al.* (1999). Leaf samples were collected at peak aphid infestation. Each genotype had three biological replicates (Pooled from 3–5 plants), each assayed in triplicate. One ml of leaf extract in methanol was taken in four ml of distilled water, 0.3 ml of 5 per cent sodium nitrite ($NaNO_2$) and 0.3 ml of 10 per cent aluminium chloride ($AlCl_3·6H_2O$). The mixture was allowed to stand for 6 minutes at room temperature. Subsequently, after adding 2 ml of 1N NaOH, the solution was diluted to 10 ml with distilled water. Finally, the absorbance of the solution was recorded at 510 nm in a Spectrophotometer (Model BGS–305, make Biogen Scientific) against a blank reagent. The results were expressed as µg quercetin equivalent/ gram dry weight.

Total flavonoid content:

$$= \frac{\text{OD 510 x Volume made up (with 80\% Ethanol)}}{\text{Aliquot taken x Weight of sample x 1000}}\ X\ 100$$

### Statistical analysis

Statistical analysis was performed to assess the relationship between flavonoid content and aphid infestations across safflower genotypes.

### Correlation

Pearson's product–moment correlation coefficient (r) was computed to determine the strength and direction of association between flavonoid content and aphid population density. The formula is mentioned below:

$$r = \frac{n\sum XY - (\sum X)(\sum Y)}{\sqrt{[n\sum X^2 - (\sum X)^2\,[n\sum Y^2 - (\sum Y)^2]}}$$

Where,
r = Correlation coefficient;
X = flavonoid content in the safflower germplasm
Y = Aphid population; n = number of observations

### Regression

Simple linear regression analysis was performed to quantify the influence of flavonoid concentration on aphid infestation parameters. Separate regression models were fitted by considering flavonoid content as an independent variable with i) Aphid population per 5cm central apical twig and ii) Aphid Infestation Index (AII) as dependent variables. Independent regression models were fitted for each response variable to avoid potential interdependence among infestation parameters. The regression equation used in the analysis is as follows:

$$Y = a+bX$$

Y = Dependent variable, a = Intercept, b = Slope,

X = Independent variable

### ANOVA

One-way analysis of variance (ANOVA) was employed to test significant differences between flavonoid content, aphid population and different genotype categories. Although there are five categories of genotypes mentioned in Table 1 based on AII, only three categories *i.e.*, Resistant (R), Moderately Resistant (MR) and Susceptible (S), were taken into consideration for analysis as none of the genotypes was classified under highly resistant (HR) and Highly Susceptible (HS).

All statistical analyses were carried out using Microsoft Excel (2021), SPSS, and Gretl. Graphical representations were used for illustrations among variables.

## Results and Discussion

The aphid infestation index (A.I.I.) presented in Table 2 demonstrated substantial variability in resistance among the evaluated safflower genotypes. None of the genotypes were identified as highly resistant. Twelve genotypes, including GMU 4167, GMU 1731, and GMU 7581, exhibited resistance to safflower aphid infestation with a population density between 18 and 28 aphids per 5 cm apical twig. Meanwhile, twenty-three genotypes displayed moderate resistance, with aphid populations ranging from 24 to 36 per 5 cm apical twig. Seventeen genotypes, including GMU 884, GMU 849, and GMU 6004, were categorized as susceptible to aphid infestation., with aphid counts varying from 38 to 52 per 5 cm apical twig section. None of the genotypes were classified as highly susceptible. The minimum flavonoid concentration was recorded in susceptible genotypes namely, GMU 2648 (13.048 µg QE $g^{-1}$ DW) and GMU 884 (13.37 µg QE/g), whereas the highest flavonoid content was detected in the resistant genotype GMU 7581 (84.76 µg QE $g^{-1}$ DW). Table 2 revealed that all 12 aphid-resistant lines contained significantly higher levels of flavonoids compared to lines that were either susceptible or moderately resistant. Genotypes possessing elevated flavonoid concentrations consistently harboured lower aphid populations had significantly fewer aphid infestations compared to the lines of germplasm that were susceptible and moderately resistant.

**Table 2:** Incidence of aphids on safflower genotypes during *rabi* 2023-24

| S.No | Genotypes | Total flavonoids in µg QE/gDW$^{-1}$ | Aphid Infestation Index (A.I.I.) | Aphid/5 cm central apical twig/plant | Category |
|---|---|---|---|---|---|
| 1 | A1 (RC) | 85.61 | 1.5 | 21.6 | R |
| 2 | GMU 7581 | 84.76 | 1.4 | 20.3 | R |
| 3 | GMU 7590 | 82.34 | 1.8 | 26.6 | R |
| 4 | GMU 6915 | 80.49 | 1.8 | 21.9 | R |
| 5 | GMU 4167 | 72.32 | 1.6 | 25.7 | R |
| 6 | GMU 1731 | 64.27 | 1.4 | 22.7 | R |
| 7 | GMU 2170 | 63.14 | 1.2 | 18.2 | R |
| 8 | GMU 2366 | 62.21 | 1.6 | 20.7 | R |
| 9 | GMU 589 | 62.21 | 1.7 | 20.7 | R |
| 10 | GMU 1067 | 59.22 | 1.2 | 21 | R |
| 11 | GMU 7942 | 58.12 | 1.6 | 23 | R |
| 12 | GMU 7943 | 57.76 | 1.3 | 18.5 | R |
| 13 | GMU 1360 | 51.23 | 2.4 | 23.3 | MR |
| 14 | GMU 7601 | 51.16 | 2.1 | 33 | MR |
| 15 | GMU 7634 | 50.5 | 2.6 | 24.3 | MR |
| 16 | GMU 7591 | 49.97 | 2.5 | 28.4 | MR |
| 17 | GMU 7572 | 49.49 | 2.9 | 24.6 | MR |
| 18 | GMU 2830 | 47.54 | 2.6 | 27.3 | MR |
| 19 | GMU 580 | 47.1 | 2.1 | 33 | MR |
| 20 | GMU 5133 | 43.13 | 2.7 | 32 | MR |
| 21 | GMU 3966 | 41.14 | 2.6 | 34.2 | MR |
| 22 | GMU 7573 | 40.31 | 2.3 | 25.4 | MR |
| 23 | GMU 4123 | 39.97 | 3 | 32.1 | MR |
| 24 | GMU 969 | 39.12 | 3 | 36 | MR |
| 25 | GMU 579 | 37.59 | 2.1 | 37.4 | MR |
| 26 | GMU 4610 | 36.43 | 2.4 | 29.7 | MR |
| 27 | GMU 4983 | 36.1 | 2.6 | 32 | MR |
| 28 | GMU 1047 | 35.4 | 2.4 | 26.5 | MR |
| 29 | GMU 1301 | 34.74 | 2.8 | 35.2 | MR |
| 30 | GMU 4965 | 34.12 | 2.2 | 33.5 | MR |
| 31 | GMU 1183 | 33.14 | 2.2 | 33.3 | MR |
| 32 | GMU 3654 | 32.68 | 3 | 33.8 | MR |
| 33 | GMU 1397 | 32.12 | 2.2 | 24 | MR |
| 34 | GMU 7633 | 30.17 | 2.6 | 37.4 | MR |
| 35 | GMU 7579 | 30.18 | 2.7 | 32.5 | MR |
| 36 | GMU 6851 | 29.16 | 3.4 | 33 | S |
| 37 | GMU 2757 | 28.95 | 3.2 | 33.1 | S |
| 38 | GMU 6037 | 27.13 | 3.1 | 29 | S |
| 39 | GMU 849 | 26.23 | 3.3 | 36.2 | S |
| 40 | PBNS12 (SC) | 25.56 | 3.8 | 47.7 | S |
| 41 | GMU 5865 | 22.3 | 3.4 | 35 | S |
| 42 | GMU 2644 | 22.13 | 3.4 | 41.4 | S |
| 43 | GMU 1440 | 21.76 | 3.1 | 34.3 | S |
| 44 | GMU 5774 | 21.76 | 3.7 | 29 | S |
| 45 | GMU 2177 | 20.95 | 4.2 | 41.2 | S |

| | | | | | |
|---|---|---|---|---|---|
| 46 | GMU 6004 | 19.42 | 3.6 | 47.3 | S |
| 47 | GMU 2131 | 19.26 | 4 | 50.5 | S |
| 48 | GMU 184 | 16.72 | 3.2 | 42.3 | S |
| 49 | GMU 3799 | 16.63 | 3.6 | 43.2 | S |
| 50 | GMU 1894 | 16.21 | 3.4 | 38 | S |
| 51 | GMU 884 | 13.37 | 3.6 | 43.2 | S |
| 52 | GMU 2648 | 13.04 | 3.2 | 33.9 | S |

(R – Resistant, MR – Moderately Resistant, S – Susceptible)

**Table 3:** Correlation coefficient between flavonoids and aphid population/5cm central twig/plant

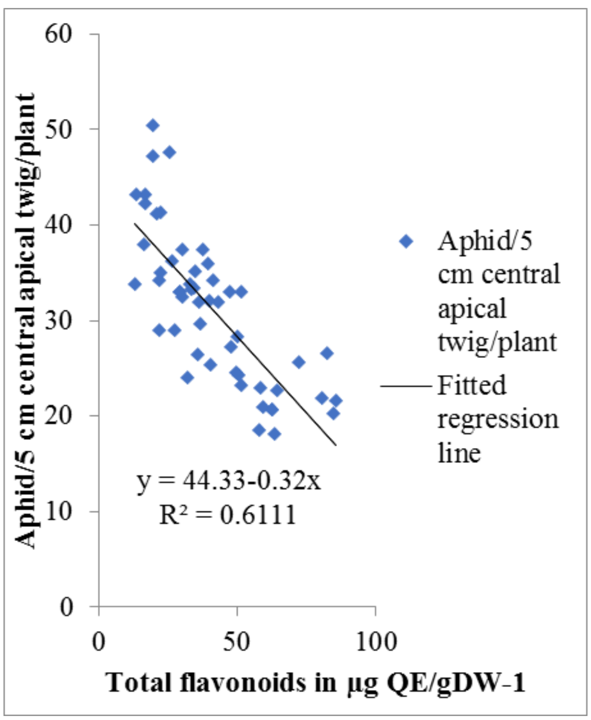
| **Values of the correlation coefficient** | |
|---|---|
| | **Total flavonoids** |
| **Aphid population/5cm central twig** | -0.78174*** |

***- Level of significance at 1% level ($p<0.001$)

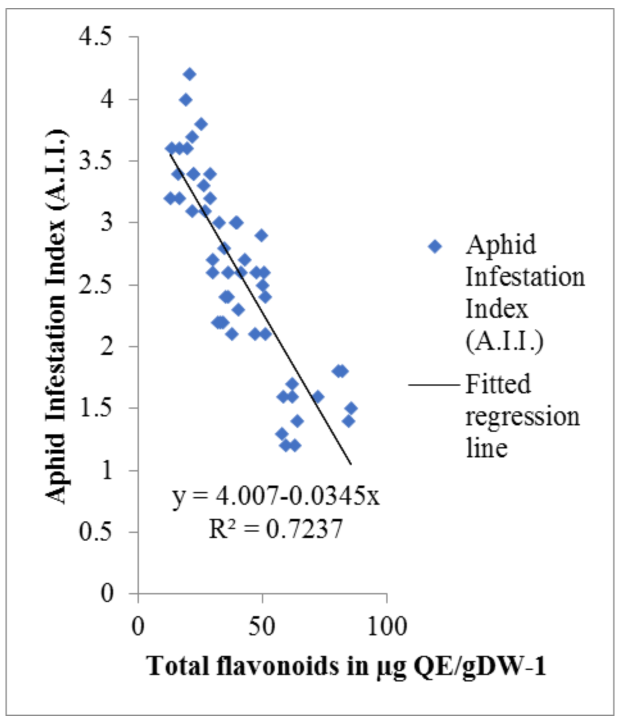
A highly significant negative correlation (Table 3) was found between total flavonoid content and the Aphid population, with a correlation coefficient of -0.78174***.

Both the linear regression models (n=52) further supported the observed correlation by revealing a significant negative effect of flavonoids on aphid population and infestation. The first model *i.e.*, regression between flavonoids and aphid population, showed a highly significant relationship ($F = 78.57$, $p < 0.001$) with a coefficient of determination $R^2= 0.611$, indicating 61.11 per cent of variation in aphid population was accounted by flavonoid content. This suggests the aphid population is substantially influenced by flavonoid concentration.

Similarly, the second regression model, relating flavonoid content to Aphid Infestation Index (A.I.I.), exhibited an even stronger association ( $F=130.96$, $p < 0.001$) with $R^2$ of 0.7237, indicating that 72.37 per cent of the variation in A.I.I. was attributable to total flavonoid content.




(i)




(ii)

**Fig. 1: (i)** Regression relationship between total flavonoid content and aphid population in safflower genotypes **(ii)** Regression relationship between total flavonoid content and Aphid Infestation Index

The one-way ANOVA revealed highly significant differences in flavonoid content among the safflower genotypes ($F=138.84$, $p < 0.001$) indicating considerable variation in flavonoid content among the experimental genotypes. The mean flavonoid content of the genotypes is presented in Table 4.

**Table 4:** Mean flavonoid content among resistant, moderately resistant and susceptible genotypes

| Category | N | Mean flavonoids ± SE (µg QE $g^{-1}$ DW) | Group |
|---|---|---|---|
| Resistant (R) | 12 | 69.95 ± 2.84 | a |
| Moderately Resistant (MR) | 23 | 39.12 ± 1.52 | b |
| Susceptible (S) | 17 | 21.34 ± 1.14 | c |

Note: Significance level at 5% according to Tukey's HSD test

Differences in flavonoid content among genotypes highlighted the possible role of flavonoids in resistance to aphid infestation. The higher the flavonoid content, the higher the resistance against aphids and vice versa.

Post hoc comparison using Tukey's Honestly Significant Difference (HSD) test ($\alpha = 0.05$) revealed a significant difference among the resistant, moderately resistant and susceptible genotypes. Based on Tukey grouping, the genotypes were classified into groups "a", "b" and "c".

The present study revealed a significant contribution of flavonoid content towards aphid resistance among different safflower genotypes. Resistant genotypes exhibited Higher flavonoid content compared to moderately and susceptible genotypes. These findings are in accordance with Srinivasa *et al.* (2023), who reported a lower aphid infestation or minimal damage in breeding lines indicting their potential against pest resistance. Similarly, Kariyat *et al.* (2019), also confirmed that maximum contents of total flavonoids were recorded in resistant cultivars and minimum content in highly susceptible cultivars. Van Chung (2018) also reported that total flavonoids in soybean leaves was highest (149.32 µg QE. $g^{-1}$ dw) in resistant cultivars and lowest content in highly susceptible cultivars.

Further statistical analysis highlighted the role of flavonoids in imparting aphid resistance. Xu *et al.* (2021) in their study investigated relationship between phenolic metabolites and aphid resistance in wheat cultivars. They observed that resistant cultivars possessed higher phenolic content along with reduced aphid susceptibility. Golawska *et al.* (2008) reported flavonoid levels of 16.40 µg QE $g^{-1}$ DW (apigenin +RCO-) in their experiment, indicating that flavonoid levels play an important role in imparting resistance against the pest. A strong negative correlation was observed between the pest infestation and total flavonoid content.

Zhang *et al*. (2025) also observed significantly suppressed feeding of *Aphis gossypii* due to higher flavonoid compounds, which was demonstrated through regression analysis. Likewise, Amit *et al.* (2021), while studying *Lipaphis erysimi* resistance in Brassica genotypes, concluded that resistant cultivars harboured comparatively lower aphid populations, emphasising the importance of biochemical constituents in aphid resistance breeding.

## Conclusion

Genotypes possessing elevated flavonoid concentrations consistently harboured lower aphid populations. All resistant genotypes possessed significantly higher flavonoid content along with reduced aphid infestation and lower Aphid Infestation Index. A significant negative correlation (-0.78174***) was observed between total flavonoid content, A.I.I. and aphid population parameters, indicating that increased flavonoid concentration was associated with reduced aphid infestation. Regression analysis further confirmed that the increased flavonoid contents reduce the aphid infestation levels.

ANOVA and Tukey's HSD test revealed a significant variability of flavonoid content among different genotype categories. Collectively, the findings establish flavonoid content as an important biochemical determinant of aphid resistance and highlight its potential utility as a selection criterion in safflower resistance breeding programmes. aimed at developing new safflower varieties with enhanced resistance to aphids.

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Conflicts of Interest: There are no conflicts among authors

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