Bio-chemical factors associated with resistance to Aphid, *Hyadaphis coriandari* (Das) in fennel at Bikaner, Rajasthan, India

Abstract

Fennel (*Foeniculum vulgare* Miller) is a significant spice crop from the Apiaceae family, often referred to as 'saunf,'. Aphid**,** *Hyadaphis coriandari* (Das) remained chief constraints in realising potential production and productivity of fennel in arid region. The biochemical characters *viz*., free amino acids, total soluble sugar and phenol content in different genotypes of fennel were responsible for resistance. The experiment was conducted using a randomized block design and involved ten different fennel varieties with three replications. The plot size was 2.4 x 3.0 meter and inter row and inter plant distance were 40 cm x 20 cm, respectively. The biochemical characters of varieties/entries *viz*., total soluble sugar, total phenols and free amino acids were estimated during peak aphid population on the crop. The free amino acids content in different varieties varied from 1.05 to 2.93 per cent. The variety RF-205 contained lowest per cent of free amino acid, harbored minimum aphid population and the RF-145 contained highest per cent of free amino acids and had highest aphid population. The RF-205 variety lowest content of TSS (0.90 mg/100g) harbored lowest aphid population (44.40 aphids/plant) and variety AF-2 contained highest 1.14 mg/100g of TSS. That shows least susceptibility against this pest. The total phenol content ranged from 2.33 to 4.55 per cent being minimum in RF-145 and maximum in variety RF-205. Total phenol (r= -0.99) were significantly negatively correlated with peak aphid population.

Key words: Bio-chemicals, Fennel, Aphid, Phenol, Insect, Resistance, Host plant resistance.

Introduction

Fennel (*Foeniculum vulgare* Miller) is a significant spice crop from the Apiaceae family, often referred to as 'saunf,' and holds a prominent position among spices in India. Fennel is extensively grown in temperate and sub-tropical regions across the globe, with significant cultivation taking place in countries in northern hemisphere. France and India are renowned as "Home of spices" (1-7). In India, fennel is grown across an area of 239.0 thousand hectares, resulting in 107.0 thousand tonnes production annually and 2233 kg per hectare, productivity rate (8). Fennel is cultivated in several states in India, including Rajasthan, Uttar Pradesh, Madhya Pradesh, Punjab, Bihar, and West Bengal. Among these states, Rajasthan holds the second position in terms of both cultivation area, covering 30.7 thousand hectares, and production, yielding 32.3 thousand tonnes of fennel, with a productivity rate of 10512 kg per hectare in the country (9) accounts for 33% of the total fennel production in India.

Insect pests pose a significant constraint to achieving higher fennel production. Among these pests, the aphid species *Hyadaphis coriandari* (Das) has been identified as a major threat to fennel crop (10-13). In addition to aphids, several other insect pests can affect fennel crops. Several pests have been reported on fennel crops, including the brown wheat mite (*Petrobia latens* Müller), thrips (*Thrips tabaci* Lindeman), whitefly (*Bemisia tabaci* Genn.), Lucerne

caterpillar (*Spodoptera exigua* Hub.), and pentatomid bug (*Agnoscalis nubile* F.). These findings were documented in studies by Nayer et al. (1982) (14), Jain (1984) (15), Kanwat (1988) (16), and Patel et al. (1986) (17). Additionally, chalcid fly, *Systoleal bipennis* Walker, thrips of the species *Thrips flavus* Schr, and the gram pod borer, *Helicoverpa armigera* Hub, have also been observed on fennel crops, although they are generally considered minor pests.

The aphid *H. coriandari* is belongs to order Hemiptera and suborder Homoptera (family Aphididae). Both the nymphs and adult aphids inflict damage by sucking cell sap from tender stems, leaves, inflorescences, and developing grains while also excreting honeydew. Their rapid reproduction rate allows them to cover the entire surface of apical shoots within a short span. As a consequence of the continuous feeding by this extensive population, the leaves undergo yellowing, curling, and eventually drying. This leads to the formation of poor and shriveled seeds.

Some fennel varieties experience fewer losses due to their specific biochemical composition, making them resistant to aphids and resulting in higher yields compared to other varieties. Utilizing resistant fennel varieties for aphid management is not only more effective but also cost-efficient. Consequently, screening was conducted to identify fennel varieties that display resistance to aphids.

Materials and methods

The experiment was conducted using a randomized block design and involved ten different fennel varieties with three replications. Each plot measured 2.4 meters by 3.0 meters, and the spacing between rows and individual plants was set at 40 centimeters and 20 centimeters, respectively. Fennel seeds were sown on October 30, 2017, and the plant-to-plant distance of 20 centimeters was achieved through thinning the crop 25 days after sowing. Eight fennel varieties were obtained from the AICRP on Seed Spices at Swami Keshwanand Rajasthan Agricultural University, Bikaner, while two fennel varieties were sourced from the National Research Center on Seed Spices, Ajmer, and were used for screening varietal resistance against fennel aphids.

Observations

The fennel varieties were subjected to natural aphid infestation, and observations on aphid populations were documented. Aphid counts were conducted in the morning on a weekly basis, starting soon after their appearance and continuing until the crop was harvested.

Additionally, the biochemical characteristics of the varieties or entries, including total soluble sugar, total phenols, and free amino acids, were analyzed during the period when aphid populations were at their peak on the crop.

Interpretation of data

The aphid population data collected from our experimental field underwent a transformation using the formula $\sqrt{X+0.5}$, as recommended by Panse and Sukhatme in 1967 (18). After this transformation, we conducted a statistical analysis using variance techniques. The categorization of fennel varieties was determined by identifying the peak aphid population

observed during the crop season, following the methodology outlined by Choudhary and Pal in 2009 (19).

$$\bar{X} \pm \sigma$$

$\bar{X}$ = mean of the peak aphid population

σ = Standard deviation

The categories were made as

I. Less susceptible
II. Moderately susceptible
III. Highly susceptible

Estimation of biochemical of fennel varieties/entries

(A) Estimation of total phenol

The assessment of total phenol content was carried out using the procedure outlined by Bary and Thorpe (20) in their work published in 1954. Initially, 500 milligrams of fresh leaf material were taken and homogenized in 5-6 milliliters of hot 80% ethanol, using a homogenizer operating at 5000 rpm for 10 minutes. The resulting supernatant was collected, and its volume was adjusted to 10 milliliters with 80% ethanol. From this solution, 0.5 milliliters were extracted and further diluted to one milliliter with distilled water. Folin's reagent (0.5 milliliters) was then added, and the mixture was allowed to stand at room temperature for three minutes. Subsequently, one milliliter of freshly prepared 20% sodium carbonate solution was added, and the total volume was adjusted to 10 milliliters with distilled water. The solution in the test tube was briefly heated by immersing it in a boiling water bath for a duration of one minute. Subsequently, the solution was allowed to cool, and we measured the absorbance at 650 nm using a UV-spectrophotometer from Systronix. A standard curve was established using known quantities of tannic acid for reference.

(B) Estimation of free amino acid

The analysis of free amino acids was carried out using the ninhydrin reagent method, a technique initially described by Moore and Stein (21) in their 1958 publication. The analysis was performed on the ethanol-soluble fraction. A suitable aliquot of 0.5 milliliters was taken in a test tube, dried in an oven at 60°C, and then allowed to cool. Subsequently, 1 milliliter of distilled water and 1 milliliter of ninhydrin reagent were added to the dried sample. The test tubes were then placed on a boiling water bath for 20 minutes. Following the cooling process, 5 milliliters of a diluent solution composed of n-propanol and water were introduced, and the absorbance was subsequently measured at 570 nm using a UV-spectrophotometer from Systronix. A standard curve was generated using known quantities of leucine, with the working standard amino acid solution at a concentration of 100 µg/ml.

(C) Estimation of total soluble sugar

To determine the ethanol-soluble sugar content, 100 milligrams of the respective leaves were utilized. They were homogenized in 80% ethanol (v/v), and the homogenate was centrifuged three times. The resulting supernatant was combined, and its final volume was

adjusted to 2.5 milliliters with 80% ethanol. Ethanol-soluble sugars were then quantified in this supernatant.

For the quantification of sugars, 0.5 milliliters of the supernatant were placed in a separate test tube and desiccated in an oven at 60°C. We determined the soluble sugar content using the anthrone reagent method, following the procedure established by Dubois et al (22) in 1951. In this process, 1 milliliter of distilled water was added to the dried material, and then 5 milliliters of freshly prepared anthrone reagent were introduced. The anthrone reagent was prepared by dissolving 2 grams of anthrone in 1000 milliliters of concentrated H2SO4. The test tubes containing the mixture were subsequently placed in a boiling water bath for 10 minutes, followed by a cooling phase.

The absorbance of the resulting brownish to dark green color was measured using a blank solution (1 milliliter distilled water and 5 milliliters anthrone reagent) as a reference at 620 nm, utilizing a SPECTRONIC-20 spectrophotometer. A standard curve was constructed using a known quantity of glucose for reference.

Result and Discussion

The biochemical characters *viz.*, free amino acids (r=0.99) were pointedly positively inter-related with peak population of aphid. (Table 1).The free amino acids content varied from 1.05 to 2.93 per cent in various varieties. The variety RF-205 contained lowest per cent of free amino acid, harbored minimum aphid population and the RF-145 contained highest per cent of free amino acids and had highest aphid population.

Table: 1 Biochemical character of different fennel varieties and their correlation with aphid population

Varieties	Peak aphid population	Total phenol (%)	Free amino acid (%)	Total soluble Sugar (mg/100g)
RF -125	65.03	3.73	1.92	0.94
RF- 145	89.05	2.33	2.93	1.10
RF- 281	74.10	3.39	2.36	1.01
RF- 178	60.23	3.97	1.69	1.02
RF- 101	50.40	4.23	1.22	0.92
RF- 157	66.71	3.61	2.17	1.05
RF- 205	44.40	4.55	1.05	0.90
RF- 143	54.00	4.11	1.43	0.98
AF- 1	75.77	3.14	2.49	0.95
AF-2	84.07	2.85	2.71	1.14
	Correlation	**-0.99**	**0.99**	**0.78**

The TSS content in different fennel varieties ranged from 0.90 to 1.14 mg/100g, respectively. The RF-205 variety lowest content of TSS (0.90 mg/100g) harbored lowest aphid population (44.40 aphids/plant) and variety AF-2 contained highest 1.14 mg/100g of TSS. That shows least susceptibility against this pest. However these biochemical components were found

significantly positively correlated (r=0.76 and =0.99 respectively) with aphid population. The relationship showed that increase of these chemicals in plants also resulted into increase in aphid population. The total phenol content ranged from 2.33 to 4.55 per cent being minimum in RF-145 and maximum in variety RF-205. Total phenol (r= -0.99) were significantly negatively correlated with peak aphid population. The relationship showed that with the increase of this chemical in plants resulted into decrease in aphid population. Malik (1988) (23) reported that the aphid fecundity was positively correlated with free amino acids. The phenols are cyclic aromatic compound having peculiar smell (24-25). The present findings in corroboration with that of Sachan and Sachan, 1991 (26) who reported higher phenol content in resistant varieties as compared to moderately resistant and susceptible mustard varieties to aphid and they also reported negative correlation with aphid population. Similarly, Kumar and Sangha, 2017 (27) reported negative correlation between aphid population and phenol also support the present findings. Jakhar et al. in 2017 (28) also noted a connection between the presence of protein, flavonoid, tannin, and phenols and the impact of *M. vitrata* on various cowpea genotypes.

Conclusion:

The biochemical characteristics of the various fennel varieties, including total soluble sugar, total phenols, and free amino acids, were determined at the point when the aphid population on the crop was at its peak. The content of free amino acids in different varieties ranged from 1.05 to 2.93 percent. The variety RF-205 contained lowest per cent of free amino acid, harbored minimum aphid population and the RF-145 contained highest per cent of free amino acids and had highest aphid population. The RF-205 variety lowest content of TSS (0.90 mg/100g) harbored lowest aphid population (44.40 aphids/plant) and variety AF-2 contained highest 1.14 mg/100g of TSS. That shows least susceptibility against this pest. The total phenol content ranged from 2.33 to 4.55 per cent being minimum in RF-145 and maximum in variety RF-205. Total phenol (r= -0.99) were significantly negatively correlated with peak aphid population.

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COMPETING INTERESTS

The authors hereby declares that they don’t have any competing interests to disclose.

Authors contribution

The research project was initiated and designed by Renu and H. L. Deshwal, who were also responsible for developing the experimental protocols and conducting the experiments. They took the lead in drafting the initial manuscript. Amar Chand and Bishana Ram contributed to data collection and management. Ankit Saini and Arvind were responsible for sourcing the necessary literature and performing data analysis. Each of the authors has examined and endorsed the manuscript's ultimate version.

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Plate- 1 Biochemical estimations in laboratory, Department of Biochemistry