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Effect of *Pseudomonas syringae* pv. *apii* on Nutritional Value, Essential Oil Profiling and Enzymatic Activity of Ajwain (*Trachyspermum ammi* L.) Seeds

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ABSTRACT

This study aimed to look at changes in nutritional value, essential oil profiling, and enzymatic activity in ajwain seed samples caused by natural seed-borne *Pseudomonas syringae* pv. *apii* (PSA) bacterial infection. The nutritional value, essential oil profiling, and enzymatic activity of three naturally infected seed samples of ajwain, with higher incidence (78.25-82.25 per cent) of *PSA* on King's medium B, and healthy seed samples were investigated. Infected seed samples had much lower moisture, fat, fibre, ash, protein, and essential oil content, while healthy seed samples (control) had significantly higher carbohydrate content. The main phytochemicals of infected ajwain seeds were adversely altered by PSA infection, as shown by GC-MS analysis of the essential oil. Peroxidase, polyphenol oxidase, and cellulase enzyme activity were considerably higher in infected seeds than healthy seeds.

***Key words*:** *Pseudomonas syringae* pv. *apii,* Ajwain seeds, GC-MS, Nutritional value, Phytochemical components, Enzymatic activity

Ajwain (*Trachyspermum ammi* L.) is an annual herbaceous medicinal crop belonging to the Apiaceae family [1]. Rajasthan and Gujrat are India's largest producers of seed spices, accounting for around 80% of the country's total output [2]. Among the seed spices, ajwain ranks top in cultivation and production in India [3]. Many medicinal properties have been reported in the essential oil extracted from ajwain, including antioxidant activity [4], nematocidal [5-6], anti-asthmatic [7], analgesic [8] and antinociceptive [9]. Many bioactive phytochemicals found in essential oil contribute to the plant's defensive function. Thymol (87.75 per cent) and carvacrol (11.17 per cent) are the predominant phenolic phytochemical components of ajwain essential oil. In contrast, p-cymene (60.78 per cent) and γ-*terpinene* (22.6 per cent) are the major non-phenolic phytochemical components [10-11]. Seed-borne plant diseases infect ajwain, posing a severe threat to seed output and quality. Diverse lipolytic, proteolytic, and pectolytic enzymes are produced by these pathogens in infected seeds, affecting the seed's lipid, protein, and carbohydrate content. Furthermore, in plant cells, host-parasite contact activates peroxidase [12] and polyphenol oxidase [13]. Considering the preceding, the current study was conducted to determine the influence of *P. syringae* pv. *apii* isolate from ajwain seeds on the nutritional value, phytochemical components, and enzymatic activity of the seed.

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MATERIALS AND METHODS

The changes in nutritional value and enzymatic activity were studied using naturally infected seed samples of Ajwain with *Pseudomonas syringae* pv. *apii* (accession no. A 1507, A 1562, and A 1637) and carrying 82.25 per cent, 80.5 per cent, and 78.25 per cent incidence of the pathogen on semi selective modified King's B medium (KBC) [14]. As a control, healthy seed samples (accession no. A 1510, A 1565, and A 1628) were used.

Analytical nutrition

The Association of Official Analytical Chemists (AOAC) 1995 standard method was used to determine the nutritional value of ajwain seeds. The nutritional values

were calculated as a percentage. The weight difference method was used to determine moisture and ash levels [15]. The fat content was determined using a solvent extraction method that employed Soxhlet equipment and anhydrous petroleum ether. The total fibre content of fat-free and moisture-free seed samples was calculated using an acid-alkali wash. Protein content was calculated by multiplying nitrogen content by 6.25 and analysing it using the Kjeldahl method, whereas carbohydrate content was calculated by subtracting 100 from (moisture + fat + fibre + ash + protein) [16]. Hydro distillation was used to extract essential oil from ajwain seeds using a Clevenger type device [17]. Three duplicates of 100g seeds were crushed using a mortar pestle and placed in a flask with 500 ml distilled water. The flask was heated in a Clevenger-style set-up for 5-6 hours until the oil was extracted entirely. Oil was collected in amber bottles from the device and dried with sodium sulphate to remove water. Following dehydration, 100 per cent pure oil was weighed and dispensed into an amber bottle to be kept refrigerated at 4°C until use.

Profiling of essential oils

The essential oil of ajwain seeds was extracted by hydro distillation, and GCMS was used to profile it (Thymol, carvacrol, p-cymene, -pinene, and limonene). A Shimadzu GCMS-QP2010 Ultra machine was used for GCMS analysis. The samples were injected in the split mode (1 l volume/sample) with a split ratio of 1:25. For separation, a 30 m x 0.25mm x 0.25m Rtx-5MS (5 per cent diphenyl – 95 per cent dimethylpolysiloxane) capillary column (5 per cent diphenyl – 95 per cent dimethylpolysiloxane) was utilised. A continuous flow of 1.0 ml/min of helium was used as the carrier gas. The oven was preheated at 60°C for 2 minutes before being raised to 260°C for 10 minutes. The ionisation energy of the Mass Spectrometer was 70 eV, with temperatures of 260°C for the interface and 280°C for the ion source. The scan range for Mas was 50-550 amu [18]. The chromatograms were analysed based on the RT (Retention time) and RI (Retention Index) of constituents, as well as a comparison of mass spectra of constituents available on the GCMS data system's mass spectral library and in NIST-MS (National Institute of Standard and Technology), to identify various phytochemical constituents.

Analysing the activity of enzymes

A colorimetric assay method [19] was used to examine changes in the enzymatic activity of polyphenol oxidase (PPO), peroxidase (PEO), and cellulase enzyme. On moistened blotter papers, healthy and naturally infected Ajwain seeds were cultured for 14 days. After that, 300 mg of these incubated seed samples were macerated in 3 ml of a 0.1M phosphate buffer solution with a pH of 6.5. This macerated seed sample was centrifuged at 10,000 rpm for 15 minutes at 4°C temperature. The supernatant was transferred to another test tube using the Pasteur pipette after centrifugation and utilised for enzyme testing [20].

Peroxidase

For the peroxidase enzyme assay, 0.2 ml of supernatant was taken in triplicate, and 0.1 ml of O-dianisidine solution (1 mg/ml in methanol) was put in a cuvette and combined with 3.5 ml of 0.1 M phosphate buffer to start the reaction (pH 6.5). The reaction mixture's cuvette temperature was regulated to 28-30°C in a continuous water bath. The cuvette was placed in a spectrophotometer set at 430 nm to record the absorbance, then 0.2 ml hydrogen peroxide (0.2M) was added to the cuvette containing the reaction mixture to record the absorbance. The initial absorbance (Ao) was recorded using a timer, followed by subsequent absorbance values every 30 seconds for up to 3 minutes. R The enzyme activity was represented in terms of increase in absorbance change in OD per unit per mg seed weight, and the graph was generated for various increasing absorbance values against time.

Polyphenol oxidase

For the polyphenol oxidase enzyme assay, 1.0 ml of supernatant was combined with 3 ml of the catechol reaction mixture (0.01 M catechol in 0.1 M phosphate buffer, pH 6.0). The colour shift caused by catechol oxidation was measured in a spectrophotometer at 495 nm for up to 5.0 minutes at 30-second intervals. The enzymatic activity was represented by rising absorbance per unit time per mg seed weight, and the graph was produced for varied absorbance values against time.

Cellulase

For cellulase enzyme, the germinated seeds were pulverised with a mortar and pestle in 3 ml of 0.1 acetate buffer (pH 5.4). To make the reaction mixture, 1 ml of enzyme extract (supernatant) was mixed with 0.5 ml of 0.1 M acetate buffer (pH 5.4), and the mixture was equilibrated at 30°C. After adding 1.5 ml of carboxymethyl cellulose to this reaction mixture, it was incubated for 2.0 hours at 30°C. After adding 3 ml of dinitro salicylic acid to the incubated mixture, it was heated for 3 hours. This combination was cooled, and the absorbance was measured at 560 nm using a spectrophotometer.

RESULTS AND DISCUSSION

Ajwain seeds are high in carbohydrate (47.54 per cent), fat (4.83 per cent), moisture (11.6 per cent), ash (11.5 per cent), fibre (4.3 per cent), protein (20.23 per cent) and essential oil (2.5-5.0 per cent) [21]. The effect of the bacterial pathogen on the nutritional value of ajwain seeds was investigated in this study (Table 1).

Nutrient content

The mean moisture content of healthy seed samples (8.79 per cent) was significantly more than the mean moisture content of naturally infected seed samples (7.09 per cent) with *Pseudomonas syringae* pv. *apii*, indicating that seed moisture content became substantially *(P≥0.01)* lower due to infection than healthy seeds (Table 1). *Xanthomonas* infection in lentils [22] and *Pseudomonas syringae* infection in cluster bean [23] seeds resulted in reduced moisture content in infected seeds. Infected seed samples of ajwain had a crude fat content (mean value) of 4.88 per cent, whereas healthy seed samples had a crude fat content (mean value) of 5.86 per cent. The standard deviation of crude fat content was 0.98 per cent, which was statistically significant (P≥0.01) [24]. Fat content was also reported earlier lower in infected seeds than healthy seeds in sunflower due to *Pseudomonas syringae* pv. *syringe.*

Infected seed samples had mean crude fibre content of 9.83 per cent, 9.07 per cent, and 9.9 per cent, while healthy seed samples had it 11.17 per cent, 11.93 per cent,

and 12.18 per cent. When compared to healthy seed samples, the mean fibre content (9.76%) of infected seed samples was considerably *(P≥0.01)* lower (11.76 per cent). Reduced fibre content may be caused by the hydrolysis of complex organic materials contained in infected). Similar outcomes were also observed after being infected with *Xanthomonas campestris* pv. *Parthenii* in *Parthenium* spp [25]. The crude protein content of infected seed samples was considerably *(P≥0.01)* lower than healthy seed samples, which could be attributable to proteolytic cleavage of proteins by the enzymes released during infection. Healthy seed samples had a total mineral content of 6.97 per cent, while seed samples were naturally infected with *P. syringae* pv. *apii* had a total mineral content of 5.37 per cent. Infected seed samples had a total ash percentage that was considerably *(P≥0.01)* lower (1.6 per cent) than healthy seed samples. Pea, soybean, and bean seeds infected with *Aspergillus parasiticus* and *Fusarium moniliforme* yielded similar outcomes [26].

The mean total carbohydrate content of infected seed samples (56.17 per cent) differed by 9.15 per cent from the mean total carbohydrate content of healthy seed samples (47.95 per cent) and was higher (*P≥0.01*). Infected seed samples (accession no. A 1507, A 1562, and A 1637) had a mean essential oil content of 2.44 per cent, whereas healthy seed samples (accession no. A 1510, A 1565, and A 1628) had a mean essential oil content of 3.5 per cent, which was substantially *(P≥0.01)* lower than healthy seed samples.

Table 1 Effect of *Pseudomonas syringae* pv. *apii* on biochemical constituents of Ajwain seeds due to natural infection

S. No.	Sample accession No	Biochemical constituents						
		Moisture (%)	Crude fat (%)	Crude fiber (%)	Crude protein (%)	Ash (%)	Total carbohydrate (%)	Essential oil (%)
Check	A 1510	9.25	5.90	11.17	18.67	7.10	47.91	3.60
(Healthy seed	A 1565	8.73	5.47	11.93	19.27	6.67	47.93	3.13
samples)	A 1628	8.40	6.22	12.18	18.0.7	7.13	48.00	3.77
	Mean	8.79	5.86	11.76	18.67	6.97	47.95	3.50
Infected seed	A 1507	6.90	4.80	9.83	16.37	5.73	56.37	2.57
samples	A 1562	7.17	5.00	9.07	15.60	5.00	58.17	2.33
	A 1637	7.20	4.83	9.90	15.93	5.37	56.17	2.43
	Mean	7.09	4.88	9.60	15.97	5.37	57.10	2.44
Deviation		-1.7*	-0.98*	-2.16*	-2.70*	-1.60*	9.15*	-1.06*

Values are the mean of 3 replicates
*Significant at 1% (P≥0.01)

Profiling of essential oils

The essential oil of the diseased seed sample (A 1507) and healthy seed sample (A 1510) of ajwain contained 23 and 21 phytochemical components, respectively, according to GCMS analysis. These components were identified and confirmed using the chemical formula, retention time (RT), and peak area from the National Institute of Standards and Technology's mass spectrum database. The RT and area percentage of all components in the essential oil of the infected seed sample and the healthy seed sample are shown in (Table 2). (Fig 1-2) show the chromatogram profile. Thymol 36.01 per cent and 39.93 per cent, carvacrol 1.14 per cent and 1.36 per cent, β -pinene 3.26 per cent and 4.87 per cent, p-cymene 0.8a per cent and 0.73 per cent, and limonene epoxide 0.42 and 0.21 per cent, respectively, were found in infected and healthy seed samples. Infected seed samples had lower thymol, carvacrol, and β -pinene levels than healthy seed samples. Infected seed samples had higher levels of p-cymene and limonene epoxide than healthy seed samples.

Table 2 Essential oil profiling of Ajwain seeds by GCMS

Compound name	Sample accession no. A 1510 (Check)			Sample accession no. A 1507 (Infected)		
	Retention time	Area	Area (%)	Retention time	Area	Area (%)
α-Thujene	4.985	8501593	0.79	4.982	10387908	0.89
α-Pinene	5.126	4888520	0.46	5.124	5914652	0.50
Cyclopentane	5.622	2572865	0.24	5.620	3189134	0.27
β- Terpinene	5.763	6344723	0.59	5.760	7697076	0.66
β-Pinene	5.869	52294063	4.87	5.867	61688026	3.26
β –Myrcene	5.990	12265960	1.14	5.987	14401513	1.23
δ -3-Carene	-	-	-	6.366	1064240	0.09
p-cymene	6.496	7793843	0.73	6.494	9762137	0.83
p-Mentha-1,5,8-triene	6.657	223006042	20.77	6.654	243058284	17.73
β –Phellandrene	6.748	4591583	0.43	6.746	5803650	0.49
Isoterpinolene	7.219	270813040	25.23	7.217	294885548	23.15
4-Thujanol	7.381	1687619	0.16	7.379	2063582	0.18
δ -4-Carene	7.628	1255216	0.12	7.626	1496954	0.13
Linalool	-	-	-	7.803	1291461	0.11
Trans sabinene hydrate	7.882	4744570	0.44	7.880	6347085	0.54
Limonene epoxide	8.848	2210089	0.21	8.846	4907278	0.42
1,6-Heptadien-4-ol, 4-propyl	8.920	3506531	0.33	8.918	6324055	0.54
Terpinen-4-ol	9.152	5212860	0.49	9.151	6644931	0.57
Isothymol	10.592	11542859	1.08	10.591	15827179	0.35
Thymol	10.826	429013344	39.96	10.828	445749648	36.01

Carvacrol	10.894	14649928	1.36	10.895	16796028	1.14
α –Santalol	15.695	4909287	0.46	15.695	5221012	0.45
trans-β-Santalol	16.165	1698383	0.16	16.164	2203418	0.19

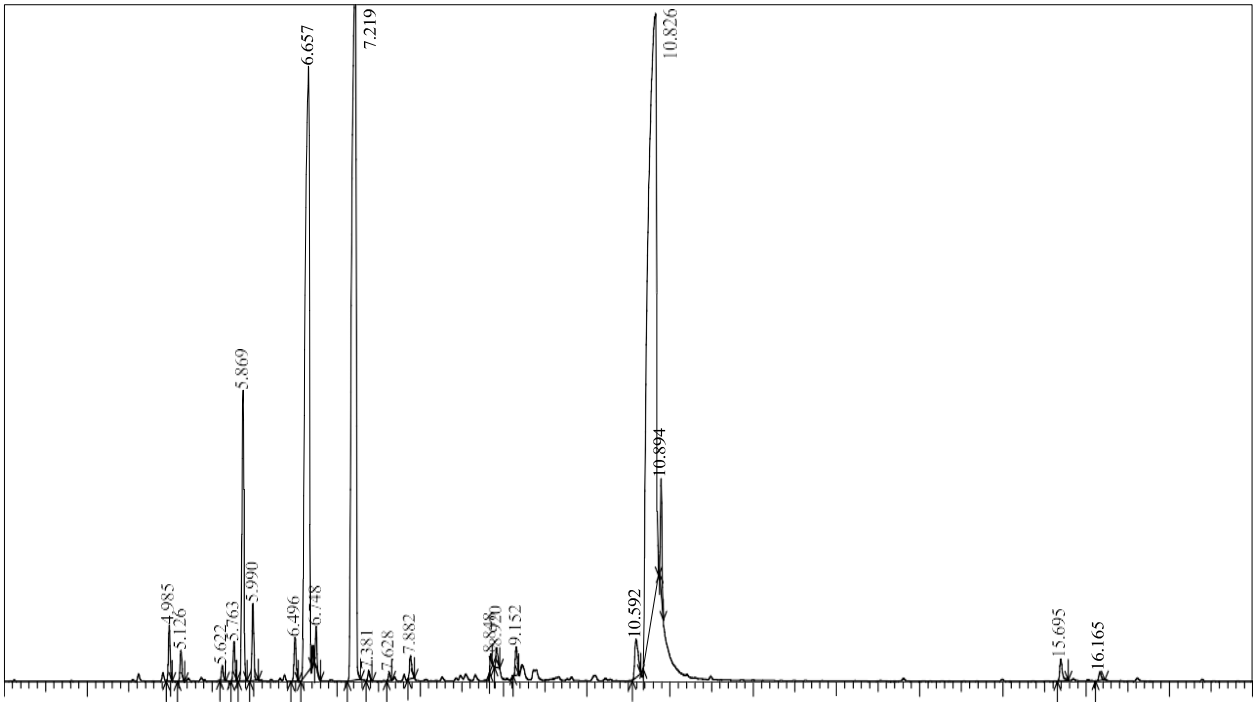

Fig 1 GCMS chromatogram of healthy seed sample of ajwain (A 1510)

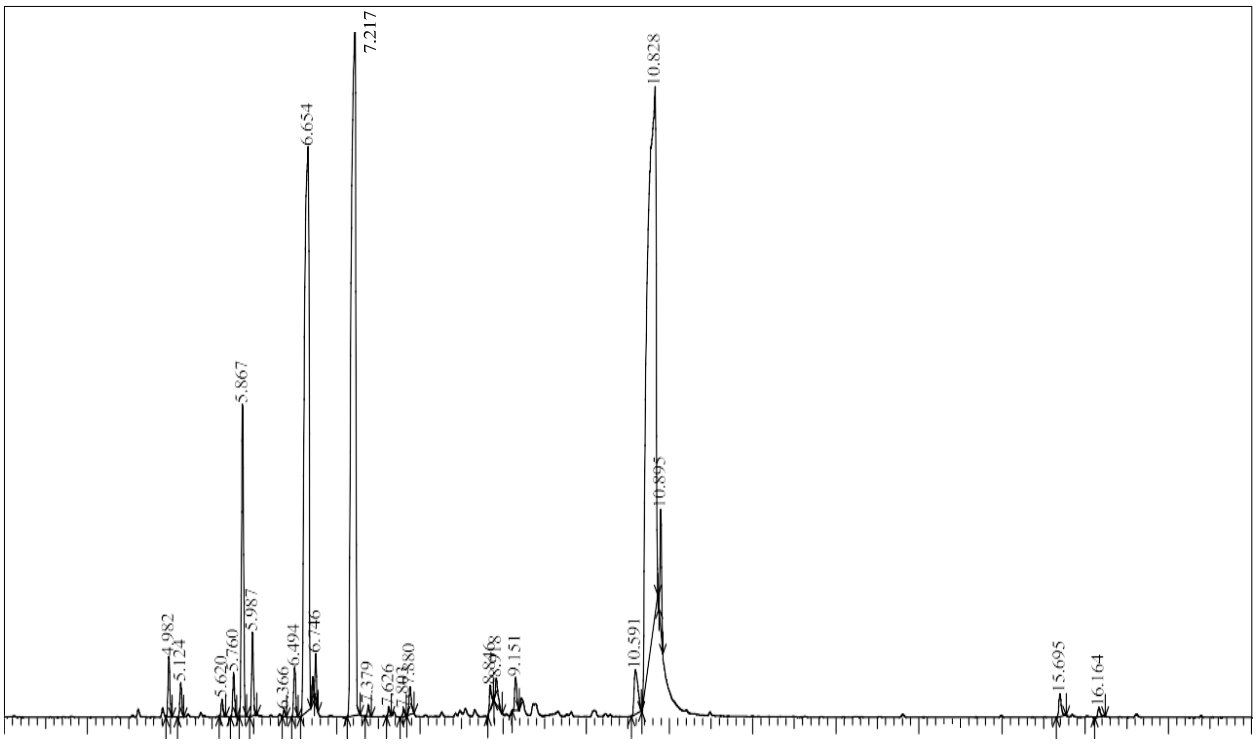

Fig 2 GCMS chromatogram of infected seed sample of ajwain (A 1507)

Activity of enzymes

When a plant cell interacts with a pathogen, the enzyme activities of the cell change primarily to play a defensive function against the infection, changes in enzymatic activity of peroxidase, polyphenol oxidase, and cellulase were identified in seed samples of ajwain naturally infected with *P. syringae* pv. *apii* as compared to healthy seeds in the current study due to the pathogen's influence. Peroxidase causes an oxidative burst of the pathogen by producing a large number of free radicals (superoxide anions) and hydrogen peroxide in infected tissue [27]. Polyphenol oxidase is a copper-containing enzyme with nuclear coding that catalyses the hydroxylation of phenols to quinines [28]. Cellulase is a hydrolytic enzyme that breaks glycosidic linkages to break down cellulose. The effect of the pathogen on the activity of enzymes is shown in (Table 3)

Table 3 Changes in enzymatic activity of Ajwain seeds infected with *Pseudomonas syringae* pv. *apii*

S. No.	Test samples	Enzymatic activity		
		Peroxidase (ΔOD/min/mg seeds)	Polyphenol oxidase (ΔOD/min/mg seeds)	Cellulase (m Mole glucose released/h)
Check (Healthy seed samples)	A 1510	0.883	0.620	0.295
	A 1565	0.666	0.736	0.338
	A 1628	0.746	0.710	0.368
	Mean	0.765	0.689	0.334
Infected seed samples	A 1507	1.039	0.987	0.654
	A 1562	0.984	1.125	0.609
	A 1637	1.228	1.061	0.520
	Mean	1.084	1.058	0.594
	Deviation (2-1)	0.338**	0.369**	0.260**

Values are the mean of 3 replicates, **significant at 1% (P≥0.01)

Peroxidase

Infected seed samples' mean peroxidase enzyme activity (1.084 O.D./min/mg) was considerably *(P≥0.01)* higher than healthy seed samples' (0.765 O.D./min/mg). Similar findings were also noted in cotton that after infection with *X. campestris* pv. *malvacearum*, which causes bacterial blight disease in cotton, the level of peroxidase in cotton increased [29]. Peroxidase enzyme activity also increased in tomato seeds infected with *P. syringae* pv. *tomato* [30].

Polyphenol oxidase

The enzymatic activity of polyphenol oxidase enzyme in naturally infected seed samples of ajwain with *P. syringae* pv. *apii* was substantially (P≥0.01) higher than the mean value (0.689 O.D./min/mg) of healthy seed samples, according to the mean values (1.058 O.D./min/mg) of observed data. These findings were comparable to those of Chitoor [31] and Shivalingaiah and Umesha [32] who found a considerable rise in polyphenol oxidase and peroxidase in the interaction between rice and the bacterial blight disease-causing *X. oryzae* pv. *oryzae*. The amount of polyphenol oxidase in infected tomato tissue with *R. solanacearum* also increased considerably [33].

Cellulase

The mean cellulase activity in healthy and infected seed samples was 0.334 and 0.594 mM glucose released/h, respectively, indicating that infected seed samples had substantially higher enzymatic activity *(P≥0.01)* than healthy seed samples. These findings matched with cotton seedlings treated with *Enterobacter asburiae* and *Pseudomonas fluorescens* that the cell wall disintegrating enzyme cellulase was higher in treated cotton seedlings than in untreated seeds [34].

CONCLUSION

The nutritional value, essential oil profile, and enzymatic activity of naturally infected seed samples of ajwain with *P. syringae* pv. *apii* were all considerably altered in the current study. Moisture, fat, fibre, ash, protein, and essential oil content of infected seeds were significantly lower than healthy seeds. However, carbohydrate content and enzyme activity (peroxidase, polyphenol oxidase, and cellulase) were significantly higher. The phytochemicals thymol, carvacrol, and β-pinene were significantly lower in infected seed samples than healthy seed samples, whilst p-cymene and limonene were significantly higher in infected seed samples compared to healthy seed samples. Thus, *P. syringae* pv. *apii* infection causes loss in nutrition quality of seeds of ajwain.

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