International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 05-12-2020; Accepted: 24-12-2020: Published: 05-01-2021
www.botanyjournals.com
Volume 6; Issue 1; 2021; Page No. 47-49

Effect on primary metabolites in cucumber seeds (*Cucumis sativus* L.) infected with *Fusarium oxysporum* f.sp. *Cucumerinum*

Poonam Arora[1], Roshan Meena[1], Dilip Kumar Sharma[2*]
[1] Botany Research Laboratory, Department of Botany, Agrawal Post Graduate College, Jaipur (Rajasthan) India
[2] Vardhman Mahaveer Open University, Kota, Rajasthan, India

Abstract
Cucumber (*Cucumis sativus* L.) is an important vegetable crop belongs to family Cucurbitaceae. It contains astringent and antipyretic properties and used to treat constipation, indigestion, jaundice and dehydration. In a field survey 102 fruit and seeds samples were collected from various cucumber growing districts of Rajasthan to know the incidence of various diseases. The fruit samples exhibit discolouration and fungal growth were used to detect the primary and secondary phytochemical changes. Two naturally infected fruit samples (lab ac no Cu-F23 and Cu-F29) revealed heavy incidence of *Fusarium oxysporum* f.sp. *cucumerinum* were selected for study. The seeds were extracted from such infected fruits; dried in shade and used for further study. A comparative analysis of primary metabolites *viz.* total carbohydrates, proteins, lipids and phenols in seeds extracts from healthy and naturally infected fruits of cucumber was carried out. The dry weight of total soluble sugar (3.1, 4.8 mg/gm), total starch (1.3, 1.4 mg/gm), protein (17.33, 18.34 mg/gm), lipids (8.55, 9.55 mg/gm) and phenols (8.65, 8.99 mg/gm) analysis showed that highest concentration of proteins was recorded in healthy seeds whereas concentration of phenols was higher noted in naturally infected seeds.

Keywords: *cucumis sativus*; primary metabolites; phytochemical; *fusarium oxysporum* f.sp. *cucumerinum*

Introduction
Cucurbits are used actively in traditional herbal remedies for various diseases. Cucumber (*Cucumis sativus* L.) of plant family Cucurbitaceae [2] is originated in India [1] and extensively cultivated crop in various countries. Various plant parts have naturally occurring phytochemical that protects the host plant against several diseases. Many primary metabolites act as precursors of many pharmaceutical compounds like antipsychotic drugs. Primary metabolites are directly involved in growth and development of plants. Carbohydrates (total soluble sugar and starch), protein, phenols and lipids are primary metabolites whereas terpenoids, alkaloids and steroids are secondary metabolites [3, 4]. Cucumber extract has bioactive like alkaloids, flavonoids, carbohydrates, glycosides, proteins, amino acids, phenolic compounds, tannins, oils, fats and saponins in a significant amount [5]. These bioactive have anti-fungal, anti-bacterial, antiviral, anti-diabetic, anti-tumour and anti-AIDS characteristics.

Material and Methods
Sample Collection
Approximately 50 fruit samples and 52 seed samples of cucumber were collected in field survey from various cucumber growing districts of Rajasthan. The survey was conducted to know the various disease incidences and their effect on phytochemical. During field surveys the sign and symptoms on the various parts of plant were recorded during crop seasons. Various methods were used to analyze phytochemical in cucumber seeds. Two fruit sample of cucumber (Lab ac no Cu-F23 and Cu-F29) naturally infected with *Fusarium oxysporum* sp. *cucumerinum* were used to detect the phytochemical changes. The seeds extracted from the infected fruits were dried under shade and powdered in sterile conditions. The healthy sample was used as control samples for comparative study.

Isolation and Quantification of Primary Metabolites
Extraction and quantitative estimation of primary metabolites was carried out by following methods:

Carbohydrates
Total soluble Sugar
The dried seed powder (50 mg each) was homogenized in pestle and mortar with 20 ml of 80% ethanol separately and left for 24 hrs. Each sample was centrifuged at 1200 rpm for 15 minutes; the supernatants were collected separately and concentrated on a water bath using the method [6]. The volume make up to 50 ml by adding distilled water and processed for further quantitative analysis.

Strach
After extraction of total soluble sugars from seed powder, the residual mass was suspended in 5 ml of 52% perchloric acid [7]. Later on 6.5 ml of water was mixed into each sample and solution was shaken vigorously for 5 minutes. 1 ml of aliquot of each sample was used for the estimation of carbohydrates using the phenol sulphuric acid method [8].

Proteins
The seed powder (50mg each) was separately mixed in 10 ml of chilled 10% trichloroacetic acid (TCA) for 30 min and kept at 4^0C for 24 hours. The mixture was centrifuged separately and supernatant was discarded. Each of the residues was again suspended in 10 ml of 5% TCA and heated at 80^0C on water bath for 30 minutes. The sample was centrifuged after cooling. Supernatants were discarded and residue dissolved in 10 ml of 1N NaOH, and kept at

room temperature for 24 hrs [9]. Each of the samples (1 ml) was taken and the total protein content was estimated using the spectrophotometer method [10].

Lipids
The 100 mg powdered seed sample was mixed with 10 ml distilled water. The mixture was transferred in to conical flask containing 30 ml of chloroform and methanol mixture (2/1: v/v) [11]. The mixture was thoroughly mixed and left overnight at room temperature in dark for complete extraction. Further, 20 ml of chloroform mixed with 2 ml of water were added and centrifuged. Two layers were separated; the lower layer of chloroform included all the lipids. The layer was separated in the pre weighed glass vials. All the water soluble substances were present in the colour aqueous layer of methanol. Later on, thick interface layer were discarded in each test sample. The chloroform layers dried in vacuum and weighed. Each treatment was repeated three times and means values were calculated.

Phenols
The de-proteinized test material (200mg each) was soaked in 10 ml of 80% ethanol for 2 hours, and left it overnight at room temperature. After centrifugation of mixture, supernatants were collected separately and sustained up to 40 ml by adding 80% ethanol. Total phenol content in each sample was estimated by spectrophotometer method [12].

Result and Discussion
Phytochemical analysis revealed that alternation in the metabolism of plant tissue at various stages of disease transmission by the pathogen. In this study the variations in phytochemical estimation due to attack of *Fusarium oxysporum* f.sp. *cucumerinum* in cucumber seeds were comparatively analyzed.

Carbohydrates
In healthy seeds carbohydrates content was higher than the infected seeds. It was recorded in 3.8 mg/gm and 4.22 mg/gm in healthy seeds of cucumber. Due to attack of pathogen it was found lower in infected seeds *viz.* 1.3 mg/gm and 1.4 mg/gm in both samples ac no Cu-F23 and Cu-F29 respectively (Fig. 2, Table 1). Similar results were reported in *Mangifera indica* [13], *Brassica compestris* [14] and sponge gourd [15]. Carbohydrates play an important role in the inactivation of pectinolytic activity, essential for pathogen [16].

Proteins
The mean protein content in the healthy seeds of cucumber was 20.84 mg/gm and 19.98mg/gm. It was recorded lower in *Fusarium oxysporum* f.sp. *cucumerinum* infected seeds *viz.* 17.33 mg/gm and 18.34 mg/gm in samples Cu-F23 and Cu-F29 respectively (Fig.2, Table 1). Low concentration of protein content in infected seeds of *Cajanus cajan* than asymptomatic seeds [17] and higher concentration of protein in infected sponge gourd due to *Colletotrichum orbiculare* than the healthy fruit was reported [15].

Lipids
The mean fat content in the healthy seeds of cucumber was 10.38 mg/gm and 10.69 mg/gm in healthy seed samples. It was recorded lower 10.24 mg/gm and 9.55 mg/gm in infected seeds of cucumber samples ac no Cu-F23 and Cu-F29 respectively (Fig. 2, Table 1). Lipolytic activity of seed-borne pathogens reduces lipid content in seeds [18, 19].

Phenols
The total phenol content was recorded slightly higher 8.65 mg/gm and 8.99 mg/gm in infected cucumber seeds than the healthy. It was 6.24 mg/gm and 5.31mg/gm in healthy seed samples (Fig. 2, Table-1).
The concentration of phenolic compounds was increased with severity of disease as compared to control. Phenolic activity increased with the process of defense mechanism [20].

Kamble and Gangwane (1987) observed similar results in infected groundnut seeds [21].

Table 1: Phytochemical estimation in seeds of cucumber

S. No	Phytochemical name		Cu -F23		Cu -F29	
			Asymptomatic (mg/gm)	Symptomatic (mg/gm)	Asymptomatic (mg/gm)	Symptomatic (mg/gm)
1.	Carbohydrates	TSS	5.4 ± 0.002	3.1 ± 0.001	7.5 ± 0.007	4.8 ± 0.003
		Starch	3.8 ± 0.003	1.3 ± 0.003	4.22 ± 0.006	1.4 ± 0.05
2.	Proteins		20.84 ± 2.17	17.33± 1.13	19.98 ±1.96	18.34 ± 1.05
3.	Lipids		10.38 ± 0.03	8.55 ± 0.05	10.69 ± 0.02	9.55 ± 0.18
4.	Phenols		6.24 ± 0.02	8.65 ± 0.02	5.31 ± 0.022	8.99 ± 0.04

Values are the mean ± SE (n=3 replicates in each group) *P <0.05, **P <0.001 compared with the control; P<0.001

Fig 1: Photo of cucumber fruits and seed powder. Healthy looking (A) and naturally infected cucumber fruits (B); dried powder of healthy seeds (C) and infected seeds (D).

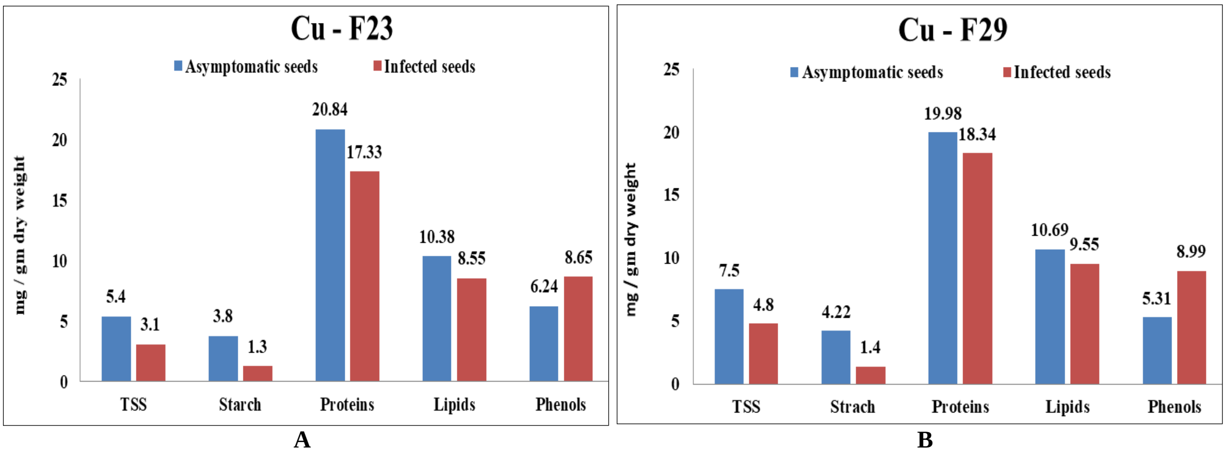

Fig 2: Quantification of primary metabolites of the healthy and infected seeds of cucumber (mg/gm dry weight)

Acknowledgment

Authors are thankful to Prof. R.L. Godharaa (Hon'ble VC, Vardhman Mahaveer Open University, Kota); Prof. Kailash Agrawal (Head of Department in Botany, Rajasthan University, Jaipur), all P.G. faculty members of Botany Department for constant support and academic guidance. Authors are also thankful to Shri Agarwal Shiksha Samiti and Principal, Agarwal P.G. College, Jaipur (Rajasthan) India for giving the lab facilities and their kind cooperation. The authors are also grateful to all the researchers whose work is referred to and couldn't recognize unconsciously and people that legitimately or in a roundabout way occupied with writing in the paper and during handy work.

References

1. Harlan JR. Our vanishing genetic resources. Science. 1975; 188:618-621.
2. Wehner TC. Breeding for improved yield in cucumber. In: J Janik (edition), Plant breeding reviews, Wiley, New York, 1989, 323-359.
3. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine: A move towards nature. Biotechnol Mol Biol Rev. 2007; 1:97-104.
4. Ramawat KG, Jain Neelam, Goyal S. Evaluation of antioxidant properties and total phenolic content of medicinal plants used in diet therapy during postpartum healthcare in Rajasthan. Int J Pharm. Sci. 2011; 3(3):248-253.
5. Gomathi S, Vijayabaskaran M, Vaijayanthimala P, Sundaram RS, Swamy SS, Kumar RS *et al.* Phytochemical Characterization of *Cucumis sativus* Linn Leaves, European Journal of Pharmaceutical and Medical Research. ISSN 3294-3211. 2016; 3(4):378-381.
6. Loomis WE, Shull CA. Methods in Plant Physiology, (McGraw Hill Book Co., New York,), 1973.
7. McCready RM, Guggoiz J, Silviera V, Owens HS. Determination of starch and amylase in vegetables. Anal. Chem. 1950; 22:1156-1158.
8. Dubois M, Gills KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1951; 28:350-356.
9. Osborne DJ. Effect of kinetin on protein and nucleic acid metabolism in Xanthium leaves during senescence. Plant Physiol. 1962; 37:595-602.
10. Lowry OH, Rose HN, Broug J, Farr AL, Randall RJ. Protein measurement with the Folin-phenol reagent. J Biol Chem. 1951; 193:265-275.
11. Jayaraman J. Laboratory Manual in Biochemistry. Wiley Eastern Limited, New Delhi. 1981, 96-97.
12. Bray HG, Thorpe WV. Analysis of phenolic compounds of interest in metabolism. Meth Biochem Anal. 1954; 1:27-52.
13. Karnawat A, Kant U. Biochemical changes in leaf gall of *Mangifera indica* L. induced by Amardiplosis brunneigallicola. Acta Botanica Indica. 1990; 18(2):312-313.
14. Singh R, Sexena VC. Studies on cauliflower wilt in relation to its post infection biochemistry. Ann. Pl. Protect. Sci. 2003; 11(1):165-167.
15. Sadda N, Varma R. Biochemical quantification of protein and its related enzymes in seeds and seedlings of Luffa cylindrica infected with Colletotrichum orbiculare. Asian J. of Microbiol. Biotech. Env. Sci. 2011; 13(3):547-549.
16. Bateman DF, Millar RL. Pectic enzymes in tissue degradation. Ann. Rev. Phytopath. 1966; 4:119-146.
17. Shukla HS, Dubey KS, Tripathi SC. Change in protein contents of Arhar (*Cajanus cajan*) due to fungal association. Legume Research. 1988; 11(2):85-88.
18. Nagel CM, Semenuiuk. Some mould-induced change in shelled corn. Pl. Physiol. 1947; 22:20-23.
19. Prasad BK. Enzymic studies of seed-borne fungi of coriander. Indian Phytopath. 1979; 32:92-94.
20. Kamble BR, Gangawane LV. Biochemical changes in groundnut at influenced by fungi. Seed research. 1987; 15:106-108.
21. Jain AK, Yadav HS. Biochemical constituents of finger millet genotypes associated with resistant to blast caused Pyricularis grisea. Ann. Pl. Protec. Sci. 2003; 11(1):70-74.