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Kinnow Plants (*Citrus nobilis* x *Citrus deliciosa*) Disease-Induced Alterations in Biochemical Composition: A comparison of Healthy and Gummosis-Infected Plants

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***Abstract*— *A economically significant citrus cultivar, the Kinnow mandarin (Citrus nobilis x Citrus deliciosa) is grown extensively throughout the Indian subcontinent, especially in northern india. In Rajasthan state, Kinnow is mainly produced in Sri ganganagar and Hanumangarh district. Kinnow plants affected by some diseases like; Citrus canker, Phytophthora, Greening (Huanglongbing), Gummosis, Citrus scab, Black fly infestation, Dieback, Anthracnose and sooty mold. Disease management is more important for decrease losses of fruit yield. A comparative analysis of the chosen biochemical parameters between healthy and diseased plants was attempted in the current study. Gummosis-infected Kinnow plants (Citrus nobilis x Citrus deliciosa) show notable biochemical alterations, according to the study, including higher concentrations of Alkaloids compounds, Phenolic, Terpenoids, Proline, MDA (Malondialdehyde) and ROS (Reactive oxygen species) and lower Chlorophyll content. According to these alterations, the disease caused a complex response in the plants that impact both primary and secondary metabolism.*

***Keywords*— Kinnow mandarin, Gummosis, Plant metabolites.**

I. INTRODUCTION

The Kinnow, a high-yielding mandarin hybrid, is widely grown throughout the Punjab region of Pakistan and India. It is a cross between the citrus cultivars king (*Citrus nobilis*) and willow leaf (*Citrus deliciosa).* Howerd B. Frost first developed it at the Citrus Research Center (University of Callifornia) Riverside in the United state. In 1915 after a long evaluation period of 20 years, it was introduced as a new variety for commercial production (Frost and Soost, 1968). Kinnow mandarin become one of the most important citrus crops in the country due to its adaptability, high productivity, and superior fruit quality (Mahajan *et al.,* 2015).

Kinnow fruits are medium to large-sized with a rich orange colour and a smooth, thin peel. The Kinnow mandarin is rich in various biochemical compounds that contribute to its nutritional value and health benefits. The fruit contains high levels of vitamin C, β- carotene, vitamin B, calcium and phosphorous (Mahawar *et al.,* 2019). They are known for their juicy segments and sweet-tart- flavor, making them popular for fresh consumption and juice production. The trees are vigorous and bear fruit early, with a tendency towards heavy and alternate bearing patterns (Singh *et al.,* 2010). The fruit's rich, sweet-tart taste with a balanced sugar-acid ratio makes it highly palatable and preferred for both fresh consumption and juice production (Ludaniya, 2008).

The kinnow mandarin possesses several unique qualities that distinguish it from other citrus fruit: high yield potential, adaptability, flavor profile, extended harvest season, post-harvest longevity. Kinnow trees are known for their prolific bearing capacity, often producing higher yields than other mandarin varieties under similar cultivation condition (Sharma and Singh, 2013). This makes them economically advantageous for farmers. The Kinnow mandarin is well-suited to a range of climatic conditions, including subtropical regions with hot summers and cool winters its adaptability extends to various soil types,

contributing to its widespread cultivation. Kinnow mandarins have a relatively long harvest period, often from December to March in the Northern Hemisphere, providing an extended market window compared to other citrus fruits With proper handling, Kinnow fruits exhibit good shelf life and can withstand transportation over long distances, which is beneficial for commercial distribution (Mahajan *et al*., 2015).

However, the high seed content can be considered a drawback in markets where consumers prefer seedless varieties. Efforts are being made through breeding programmes to develop low-seed or seedless Kinnow variants to meet consumer demands (Singh *et al*., 2017).

Plant diseases pose significant economic challenges to Kinnow cultivation in india, affecting both income of the farmers and the broader agricultural economy. Citrus disease cause substantial economic losses worldwide through reduced yields, increased production costs, and diminished fruit quality. Managing these diseases often requires significant investment in control measures, which increases the cost of production (Gottwald *et al*., 2007).

Kinnow plants affected by some diseases like; Citrus canker, Phytophthora, Greening (Huanglongbing), Gummosis, Citrus scab, Black fly infestation, Dieback, Anthracnose and sooty mold. Gummosis is the one of Devastating disease affecting kinnow fruit yield. Gummosis, a disease affecting Kinnow or citrus trees, is primarly caused by Phytophthora fungi and characterized by the oozing of gum from the trunk and branches, effective management involves a combination of cultural practice, biological control, and chemical treatment (Erwin and Ribeiro, 1996).

Kinnow plants, like other citrus varieties, exhibit various physiological and biochemical changes when affected by diseases. The plants are continuous exposure to microoraganism in the environment, as a result, have developed complex defense mechanism to recognize and protect themselves against potential pathogens. Photosynthesis and other basic metabolism related processes that are vital to plant growth are downregulated as a result of these reactions (Rojas *et al*., 2014). According to Piasecka et al. (2015) and Sudha & Ravishankar (2002), secondary metabolites in plants are essential for their defense against a variety of biotic and abiotic stresses. Plants innate immunity response to pathogen infections frequently involves an increase in the synthesis of protective secondary metabolites. These substances may have a role in regulating immunological responses such callose deposition and programmed cell death.

The goal of the current study to conduct a comparative biochemical analysis of Gummosis-infected and healthy Kinnow plants. It involve quantify and compare the amounts of primary and secondary metabolites in infected and healthy Kinnow plants. According to the research work, the primary and secondary metabolite profile of Kinnow plants will change significantly as a result of Gummosis infection. This work attempts to advance our knowledge of the Kinnow plants response to Gummosis disease by clarifying these disease-induced alteration.

II. MATERIAL AND METHODS

2.1 Study area

Six marked Kinnow orchards (sample sites) were used for the study, which was carried out in Sri Ganganagar, Rajasthan, India. The Kinnow mandarin (*Citrus nobilis* x *Citrus deliciosa*) plants that were both healthy and diseases with gummosis were selected from these orchards. Visually inspection of characteristic oozing of the gum from the trunk or branches were used to confirm the condition prior to sampling (Adesemoye *et al.* (2014).

2.2 Sample Collection

At each sampling location, leaf samples were taken from both healthy and diseased plants (10 leaves) to ensure that the plant ages and leaf positions were similar. additionally, samples were marked and transferred to an ice box in the field. Until further investigation, the leaves were kept in the labs refrigerated facility at -12ºC to prevent water loss and biochemical alteration. Glass homogenizer was used to homogenize samples before each biochemical test.

2.3 Biochemical Analysis in Kinnow plant (Citrus nobilis x Citrus deliciosa)

2.3.1 Total Chlorophyll Content

The method described by Lichtenthaler and Buschmann (2001) was used to determine the total chlorophyll content. In this process, 0.1 g of fresh leaf tissue was blended with 10ml of 80% acetone. The homogenate was centrifuged at 3,000 rpm for 5 minutes, and the absorbance of the supernatant was recorded at 663nm and 645 nm with a UV-visible spectrophotometer.

2.3.2 Total Phenolic Content

The Folin-Ciocalteu method was used to measure total phenolic content (Singleton *et al*., 1999). Fresh leaf samples (0.5g) were extracted with 80% methanol, and the extract was reacted with Folin-Ciocalteu reagent. Absorbance was measured at 765nm, and outcomes were expressed as µg of gallic acid equivalent per gram of fresh weight.

2.3.3 Protein Content

The Bradford method (Bradford, 1976) was utilized to measure protein content. Fresh leaf tissue (0.5 g) was

homogenized in phosphate buffer (pH 7.0), and the solution was treated with Bradford reagent. Absorbance was measured at 595nm, using bovine serum albumin as a standard.

2.3.4 Total Alkaloid Content

The total alkaloid content was determined using the technique described by Sreevidya and Mehrotra (2003). Ethanol was used to exract fresh leaf samples, and the resulting exract was treated with Bromocresol Green solution. Absorbance was measured at 470nm.

2.3.5 Terpenoid Content

The terpenoid content was determined using the method Ghorai *et al*. (2012).fresh leaf samples were extract was combined with Vanillin-sulfuric acid reagent. Absorbance was recorded at 608nm.

2.3.6 Proline Content

The Ninhydrin method (Bates *et al.,* 1973) was used to assess proline content. Fresh leaf samples (0.5 g) were blended in Sulfosalicylic acid and treated with acid ninhydrin. The absorbance was measured at 520nm.

2.3.7 Malondialdehyde (MDA) Content

Lipid peroxidation was evaluated by determing MDA levels via the Thiobarbituric acid (TBA) technique (Heath and Packer, 1968). Fresh leaf tissue (0.5 g) was homogenised in trichloroacetic acid and then centrifuged. The supernatant was mixed with TBA and heated. Absorbance was recorded at 532 nm and 600nm.

2.3.8 Reactive Oxygen Species (ROS)

Reactive Oxygen Species (ROS) were considered in terms of the level of H_2O_2 present. The H_2O_2 levels, which indicate ROS were determined using the method of Valikova *et al.* (2000). Fresh leaf samples (0.5 g) were homogenised in trichloroacetic acid and spun in a centrifuge. The supernatant was mixed with Potassium phosphate buffer and Potassiun iodide. Absorbance was measured at 390nm.

III. OBSERVATION

The following finding was obtained by comparing the biochemical parameters of Gummosis-infected and healthy Kinnow plants (Table-1). The eight biochemical parameters were recorded at six distinct sampling locations (Daulatpura, 7 F, Koni, Mirzawala, Madera, Fatuhi) using both Gummosis-infected and healthy plants.

Table -1 Biochemical Parameters in healthy Kinnow plants and Gummosis diseased Plants at different sampling sites.

S.N.	Biochemical Parameter	Study Site ►	Daulat-pura	7F	Koni	Mirzawala	Maderan	Fatuhi
1.	Total Chlorophyll Content (µg/g)	Healthy plant	675	715	742	752	702	682
		Diseased plant	452	512	556	426	516	612
2.	Total Phenolic Content (µg GAE/g)	Healthy plant	34.16	42.26	45.6	38.26	32.65	42.5
		Diseased plant	45.65	58.56	52.56	39.65	38.65	43.55
3.	Protein Content (µg/g)	Healthy plant	1.2	1.1	1.56	2.1	1.5	1.6
		Diseased plant	1.65	1.2	1.59	1.85	1.68	1.89
4.	Total Alkaloid Content (µg/g)	Healthy plant	2.3	2.4	2.5	2.2	2.8	3.1
		Diseased plant	3	3.5	3.1	3.2	3.5	3.5
5.	Terpenoid Content (µg/g)	Healthy plant	845	756	856	721	722	765
		Diseased plant	892	865	859	813	810	812
6.	Proline Content	Healthy plant	3.85	4.56	3.65	4.65	4.98	3.59

	(µg/g)	Diseased plant	5.65	6.6	5.6	8.56	5.65	4.65
7.	Malondialdehyde (MDA) Content (µg/g)	Healthy plant	0.01	0.02	0.02	0.01	0.02	0.03
		Diseased plant	0.02	0.03	0.03	0.02	0.02	0.04
8.	Reactive oxygen species (ROS) (µmol/g)	Healthy plant	1.6	2.15	1.82	2.59	1.85	1.66
		Diseased plant	2.65	3.56	3.12	3.65	3.58	3.5

IV. RESULT AND DISCUSSION

The research investigated the biochemical changes in Kinnow plants induces by Gummosis disease, comparing important biochemical parameters between healthy and diseased plants. (Table-1) the data show notable changes in various biochemical compounds suggesting a strong influence of the disease on the metabolic functions of plants (Figure-1, 2,3,4) additionally, these increased parameters were analyzed for statistical significance using a t-test (Table-2).

Table -2 Average value of Biochemical Parameters and their statistical significance test

S.N.	**Biochemical Parameter**	**Plants (Healthy/diseased)**	**Average value**	**Conclusion**
1.	Total Chlorophyll Content (µg/g)	Healthy plant	711.3 ± 12.75	Highly significant difference ($p \le 0.001$)
		Diseased plant	512.3 ± 27.66	
2.	Total Phenolic Content (µg GAE/g)	Healthy plant	39.24 ± 2.085	Non-significant difference
		Diseased plant	46.44 ± 3.163	
3.	Protein Content (µg/g)	Healthy plant	1.510 ± 0.1441	Non-significant difference
		Diseased plant	1.643 ± 0.1007	
4.	Total Alkaloid Content (µg/g)	Healthy plant	2.550 ± 0.1384	significant difference ($p \le 0.01$)
		Diseased plant	3.300 ± 0.0930	
5.	Terpenoid Content (µg/g)	Healthy plant	777.5 ± 24.23	significant difference ($p \le 0.05$)
		Diseased plant	841.8 ± 14.24	
6.	Proline Content (µg/g)	Healthy plant	4.213 ± 0.2406	significant difference ($p \le 0.01$)
		Diseased plant	6.118 ± 0.5495	
7.	Malondialdehyde (MDA) Content (µg/g)	Healthy plant	0.01833±0.003073	Non-significant difference
		Diseased plant	0.02667±0.003333	
8.	Reactive oxygen species (ROS) (µmol/g)	Healthy plant	1.945 ± 0.1509	Highly significant difference ($p \le 0.001$)
		Diseased plant	3.343 0.158	

4.1 Chlorophyll content

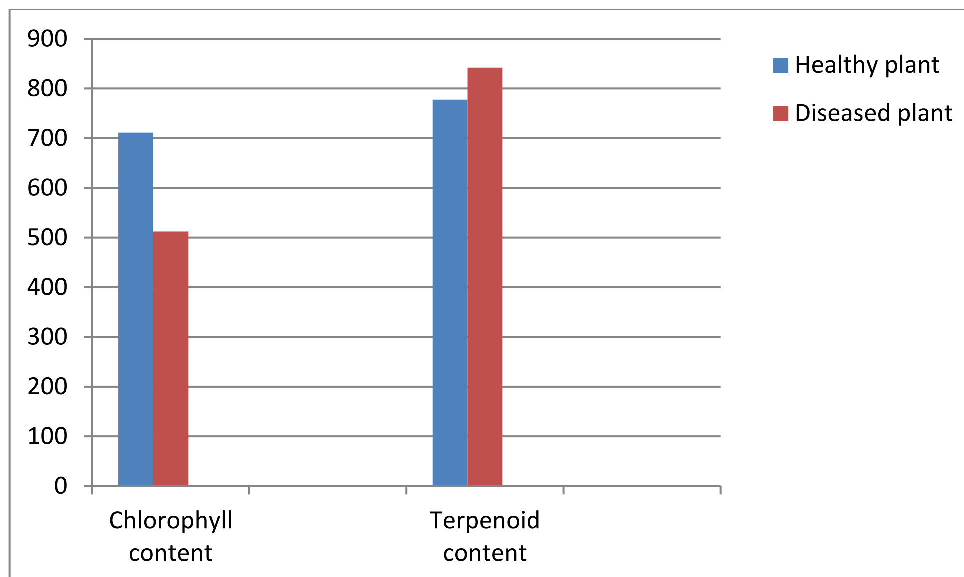

Fig.1: Alteration in Biochemical Parameter (Chlorophyll and Terpenoid content) of healthy and diseased plants

Diseased plants had a much lower total Chlorophyll content (512.3 µg/g) than healthy plants (711.3 µg/g), according to the study the significant drop in chlorophyll content marked to a sharp decline in photosynthetic efficiency, which is a typical sign of stress in plants attacked by diseases. Gummosis infection- induced oxidative stress and cellular damage may be the cause of the drop in chlorophyll content.

4.2 Protein, Alkaloid, Proline, and ROS

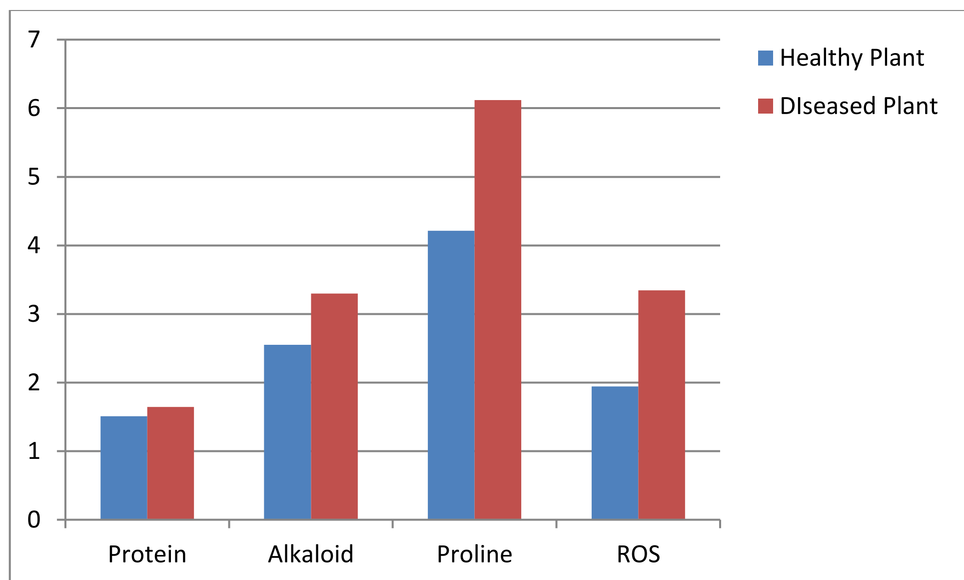

Fig.2: Alteration in Biochemical Parameter (Protein, Alkaloid, Proline, and ROS) of healthy and diseased plants

There were very slight variation in the Protein concentration between the diseased plants (1.643 µg/g) and the healthy plants (1.510 µg/g). The slight alteration seen in the investigation signifies that the Gummosis disease had minimal change on the overall synthesis or degradation of proteins, suggesting that fundamental metabolic processes remained largely unaffected by the diseases.

An increase in Alkaloids, which are secondary metabolites frequently linked to plant defense, indicate an active biochemical reaction to pathogen invasion. The study found that diseased plants had a significantly higher total alkaloid

content (3.300 µg/g) than healthy plants (2.550 µg/g). The higher Alkaloid content in diseased Kinnow plants points to an activated defence system mechanism that might help the plant resist the infection or slow its spread.

Similarly, the Proline content was significantly higher in diseased Kinnow plants (6.118 µg/g) than in healthy plants (4.213 µg/g). Adverse environmental condition and pathogen attack tend to raise Proline levels. Diseased plants increased Proline levels suggested stress responses, which might have helped the plant handle the oxidative damage brought on by the infection.

According to the study, diseased plants had considerably higher levels of Reactive oxygen species (3.343 µmol/g) than healthy plants (1.945 µmol/g). Reactive oxygen species (ROS) are frequently generated in reaction to pathogen infection and can cause damage. Reactive oxygen species are also trigger signalling pathways for defense mechanism. The higher reactive oxygen species levels in diseased plants show reactive oxygen species function as both harmful and signalling agents in the plants response to Gummosis infection.

4.3 Malondialdehyde (MDA)

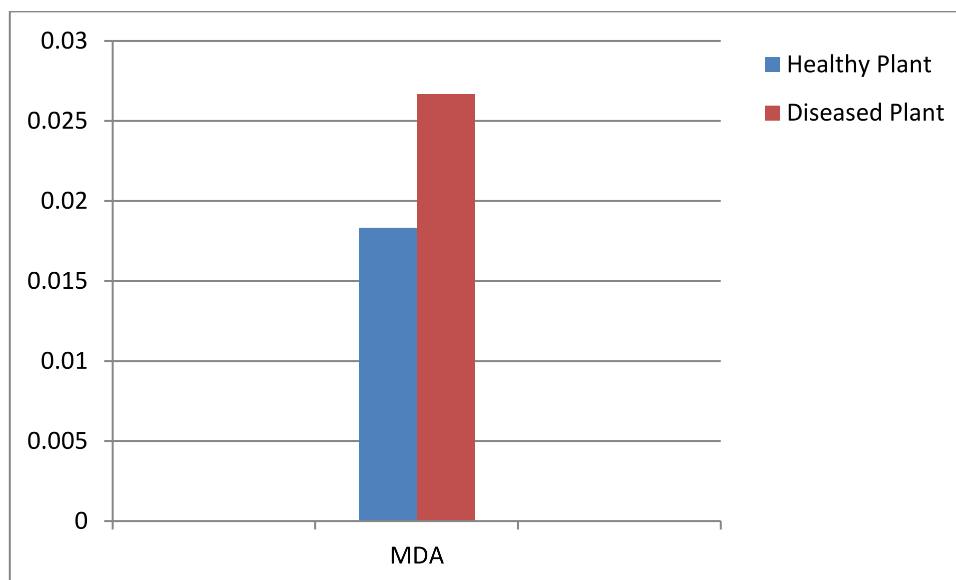

Fig.3: Alteration in Biochemical Parameter (MDA-Malondialdehyde) of healthy and diseased plants

Diseased plants had a slightly higher Malondialdehyde content (0.02667 µg/g) and healthy Kinnow plants (0.01833 µg/g). The non-significant difference in this study, implies that lipid peroxidation may not be the predominat response in plants to Gummosis infection.

4.4 Phenolic content

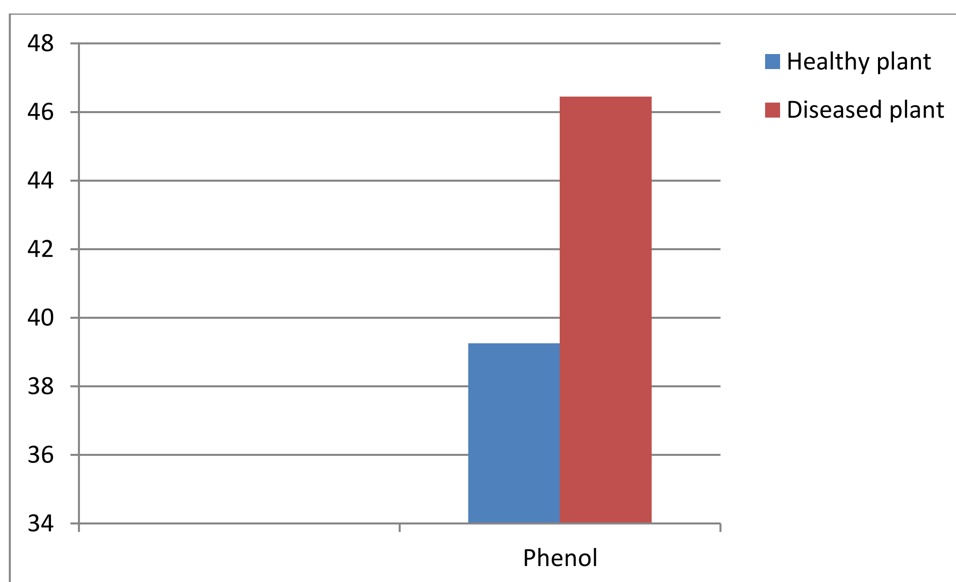

Fig.4: Alteration in Biochemical Parameter (Phenol content) of healthy and diseased plants

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The total phenolic content was found to be higher in diseased plants (46.44 µgGAE/g) than in healthy plants (39.24 µgGAE/g). Phenolics are defence compounds that help to strengthen cell walls and prevent the infection of pathogen. An active defence response may be indicated by the elevated phenolic content in diseased plants, which could strengthen resistance to the gummosis infection.

V. CONCLUSION

The comparison of biochemical compounds between Gummosis diseased and healthy Kinnow plants illustrate the diseases widespread effect on plant metabolism. The considerable decrease in chlorophyll content suggest that photosynthesis activity decreases. Increased concentration of secondary metabolites (Alkaloids, Phenolics, Terpenoids) that emphasize an improved defense response. Reactive oxygen species (ROS) and Proline concentration higher in diseased Kinnow plants. They are responsible for trigger signalling pathways and defense mechanism in plants biochemical alteration induced by Gummosis disease in Kinnow plants, providing information that may guide future disease management.

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