Journal of Stress Physiology & Biochemistry, Vol. 16, No. 2, 2020, pp. 22-31 ISSN 1997-0838

ORIGINAL ARTICLE

OPEN ACCESS

Effect of Biotic Stress on Vitamins and Nutrients of Ficus palmata

Sarvjeet Singh[1] and Sujata Bhattacharya[2]

[1] *Panjab University Chandigarh, 160014, India*

[2] *Shoolini University of Biotechnology and Management Sciences Bajhol, Solan (H.P.) 173229, India*

*E-Mail: ***sujata0685@gmail.com***

Received January 17, 2020

Biotic stress mediated effects on amount of tocopherol, ascorbic acid and nutrients like sodium and potassium ion of *Ficus palmata* were investigated in the current study. Present study revealed that the amount of tocopherol in healthy leaf was greater than diseased leaf. However, the amount of ascorbic acid in disease leaf (0.406±0.0108) was more as compare to healthy leaf (0.447±0.0386) of *F. palmata*. The amount of tocopherol in healthy leaf was 2.0915±0.4188 and in diseased leaf it was 1.786±0.1383.On the other hand, the amount of sodium in both healthy and diseased leaf was similar (1.333±0.333).The amount of potassium in healthy leaf was more (42.00±2.081) as compared to the diseased leaf (33.33±10.929). Greater the amount of ascorbic acid and tocopherol in plants more will be radicle scavenging activity and also helpful in pharmacological industry, drug production and health care.

Key words: Medicinal Plants, Biotic Stress, Tocopherol, Ascorbic Acid, Nutrients

Medicinal plants represent a rich source form which antimicrobial agents may be obtained. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al*., 1996). The world is rich with natural and unique medicinal plants. Medicinal plants are now getting more attention than ever because they have potential of myriad benefits to society or indeed to all mankind, especially in the line of medicine and pharmacological. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmoladun *et al*., 2007). The use of herbal medicine for the treatment of diseases and infections is as old as mankind. Medicinal plants play a significant role in providing primary health care services to rural people and are used by about 80% of the marginal communities around the world (Prajapati and Prajapati, 2002; Latif *et al*., 2003; Shinwari *et al.*, 2006). Each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals. These nutrients are essential for the physio-logical functions of human body. Such nutrients and bio-chemical like carbohydrates, fats and proteins play an important role in satisfying human needs for energy and life process (Hoffman *et al*., 1998; Mathews *et al*., 1999; Dingman, 2002).

Plant diseases are caused by biologically different agents (i.e., bacteria, fungi, oomycetes, and viruses), and traditionally it is considered that these agents operate, irrespective of their taxonomic affiliation, using essentially two different strategies, namely biotrophy and necrotrophy. Many apparently combine these strategies as hemibiotrophs (Glazebrook, 2005). Adverse effects on plants caused by other living organisms, including fungi, viruses, parasites, bacteria, insects, weeds and competing plants. However, recent studies have revealed several molecules, including transcription factors and kinases, as promising candidates for common players that are involved in crosstalk between stress signalling pathways. Some plants may be injured by a stress which means that they exhibit metabolic dysfunctions. Stress depends on the time period if the stress is moderate or for short period then it be recovered by plant. If the stress is severe enough, it prevents flowering, seed formation and induce senescence which leads to death of plant. Biosphere consists of a complex array of biotic communities and abiotic factors. Biotic assemblages of plants, animals and microbes interact with their physical environment in such a manner that there is a smooth flow of energy leading to clearly defined trophic structure, biotic diversity and material cycle within a system. The co-existence of different species in any ecosystem is, therefore, the outcome of interactions (flow of energy) among themselves under the influence of prevailing environmental realms. Appearance and existence of any plant community is entirely a subject of its floristic composition (represented in the form of a complete list of plants) and life from spectrum of its individual components. The life form spectra are said to be excellent indicators of micro and macroclimate (Shimwell, 1971).

Biotrophs rely on living plant tissue, whereas necrotrophs kill plant cells to derive nutrition and hemibiotrophs usually have an initial endophytic or biotrophic phase and later become necrotrophic. Mutation in key genes regulating defences may affect resistance against biotrophs and necrotrophs differently and can have a major effect on overall plant fitness (Glazebrook, 2005). Natural disease resistance is an observed phenotype in which a pathogen is less able to cause disease on one host compared to another. Several distinct phenomena are represented that can operate simultaneously as well as at different phases in the infection and development pathway. Resistance can lie at the penetration stage (e.g., the wax layer, cuticle, or cell wall) in the ability of a fungal pathogen to assimilate enough nutrients to be able to proliferate in the tissues or sporulate and spread. Resistance can be constitutive or induced, and it has been demonstrated in several plant species that induced resistance can be regulated by different signalling pathways (Van Loon, 2000).

Plants are mostly affected by Black Spot, Leaf Spot, Powdery Mildew, Rust. These diseases caused by the pathogenic stains of fungi. Since this disease removes essential nutrients from the plant, leaves may become

yellow, stunted or drop off prematurely. spores travel through the air and land on plants, spreading the fungal disease (Fig. 1, Fig. 2).

Anthracnose is often attributed to *Colletotrichum* spp. Greasy appearing spots yellow and then dies and has a halo. On leaves, mosaic spots are distinctive, contrasting with normal green colour of the foliage. The margins of the yellow spots blend gradually through a light yellow colour into the dark green of healthy tissue. Mosaic spots or lesions may be scattered uniformly over the surface of the leaves.

Common types of Diseases caused in *Ficus*

1) Anthracnose

Greasy-appearing spots yellow and then die and have a yellow halo caused by *Glomerella* (*Colletotrichum*).

2) Bacterial Leaf Spot

Angular yellow spots are limited in size by veins. Spots become brown and leaves fall. Caused by *Xanthomonas campestris*.

3) Branch Dieback

Leaves wilt, die, and fall. Small and eventually large branches die. Wood under the bark is black.

4) Cold Injury

Mature leaves have large brown blotches. Young leaves appear puckered or distorted and brown in temperatures below 40° F.

5) Foliar Nematode

Areas between the leaf veins yellow and die. Caused by Aphelenchoides

6) Leaf drop in *Ficus Benjamin* leaves yellow and fall. Caused due to Low soil moisture and low relative humidity.

*Ficus palmata*Forsk. commonly known as ‘Fegra Fig’ belongs to the family of Moraceae or Urticaceae. It is found to be growing wild in the Himalayan region, so also named as Wild Himalayan fig and is mainly the native of North-Western India and Rajasthan regions. Fegra plants are of common occurrence at places up to 1,000 metres above the sea-level. These trees are occasionally found in the forests, but grow well around the villages, in wastelands, fields (Parmar and Kaushal, 1982; Chopra *et al*., 1986).

There are numerous reports of fungal symbionts conferring tolerance to stress to host plants and disease (Bacon and Hill, 1996; Rodriguez*et al*., 2008). Throughout evolutionary time plants have been confronted with various abiotic and biotic stresses. Lacking any form of locomotion, plants have depended on seed dispersal, vegetative growth, and complex physiology either to escape or to mitigate the impacts of stress. All plants are known to perceive and transmit signals, and respond to stress such as drought, heat, salinity, and disease (Bohnert *et al.*, 1995; Bartels and Sunkar, 2005). Plants undergoes in necrosis during attacked by fungal or pathogenic factor and made different layers on foliage part of plant for differentiate the foliage region and nutrients and vitamins are soaked off from that region to healthy region of plant.

Vitamin E’s main function in the Plant body to work as an antioxidant, scavenging loose electrons—so-called “free radicals”—that can damage cells. (Institute of Medicine, 2000). Tocopherols (vitamin E) are a major lipid soluble antioxidant present in the PUFA-enriched membranes of chloroplasts and are proposed to be an essential component of the plastid antioxidant network. ROS generated as byproducts of photosynthesis and metabolism re potential sources of lipid peroxidation in plant cells, and though direct experimental evidence is lacking, tocopherols are assumed to function similarly to animals in limiting ROS damage to plant lipids. Tocopherol levels increase in photosynthetic plant tissues in response to a variety of abiotic stresses (Munne-Bosch and Alegre, 2002),

Ascorbic acid (vitamin C) in plant chloroplasts is known to help prevent a reduction in growth that plants experience when exposed to excessive light—phenomenon called photo inhibition. Vitamin C is a potent electron donor and reducing agent and also acts as water soluble antioxidant; Vitamin C helps to maintain DNA, proteins, lipids, enzymes and other antioxidants in their normal form. It does this by scavenging oxygen and nitrogen radicals and reducing metal ions (Carr and Feri, 1999).

Reactive oxygen species (ROS) are class of highly reactive molecules derived from the metabolism of oxygen. Rapid production of free radicals may lead to oxidative damage to biomolecules and results in disorders, cancer, diabetes, neural disorders and ageing. These free radicals occur in the body during an

imbalance between ROS and antioxidants such as vitamin C, vitamin E, polyphenols etc. Primary sources of naturally occurring antioxidants are grains, vegetables and fruits. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids have been recognized as having the potential to reduce disease risk (Prior and Cao, 2000). The intestinal absorption of iron is greatly increased by adequate Vitamin C. Vitamin C is present in freshest fruits and vegetables (Pao and Warner, 1993).

The amount of K present in the cell determines how many of the enzymes can be activated and the rates at which chemical reactions can proceed. Thus, the rate of a given reaction is controlled by the rate at which K enters the cell (Van Brunt and Sultenfuss, 1998). When K moves into the guard cells around the stomata, the cells accumulate water and swell, causing the pores to open and allowing gases to move freely in and out. When water supply is short, K is pumped out of the guard cells. The pores close tightly to prevent loss of water and minimize drought stress to the plant (Thomas and Thomas, 2009). When plants are deficient in K, proteins are not synthesized despite an abundance of available nitrogen (N). Instead, protein “raw materials” (precursors) such as amino acids, amides and nitrate accumulate. The enzyme nitrate reductase catalyses, the formation of proteins and K is likely responsible for its activation and synthesis (Patil, 2011). Many halophytic plants are able to take advantage of this close similarity between Na and K, and have adapted to grow in areas of high salt (NaCl) (see review by Glenn *et al*., 1999) where other less well adapted plants (i.e., glycophytes) are limited in growth due to high salinity stress (Greenway and Munns, 1980). Growth responses of halophytes to Na under saline conditions reflect the need for an osmoticum during osmotic adjustment to salinity stress (Flowers and Lauchli, 1983; Glenn *et al*., 1996).

Aim and objectives:

Present study aims at to check the impact of biotic stress on Vitamin C (ascorbic acid), Vitamin E (tocopherol) and nutrients like sodium and potassium of *Ficus palmata*. Following were the objectives of present research work:

1. To investigate the impact of biotic stress on the content of ascorbic acid and tocopherol.
2. To study the variation in the content of nutrients like Na and k upon exposure to biotic stress.

MATERIALS AND METHODS

Plant used in study

Ficus is the genus of the family *Moraceae* that comprises about 800 species (Harrison, 2005). Most of the members of the family are very high trees, shrubs and rarely herbs often with milky juice (Hutchinson *et al*., 1958). *Ficus palmata* is herbaceous perennial plant however the fruits are also used as a dry vegetable. *Ficus palmata* is a very tasty fruit. It is very much liked by all, the fig is a very juicy fruit and taken on standardizing the techniques for making various products such as squash, jam and jelly from this fruit (Saklani and Chandra, 2012). *Ficus palmata* plant is used in various disease e.g. gastrointestinal, hypoglycemic, insulinase, anti-tumor, anti-ulcer, anti-diabetic, lipid lowering and antifungal activities.

Chemicals used

Chemicals used in this study are methanol, ethanol, Trichloric acetic acid (TCA), Sulphuric acid, activated carbon etc.

Collection of medicinal plant

The plant part of *Ficus palmata* has been used in the present study and they were collected in November 2017, from the Solan (Manjoli) area of Himachal Pradesh. The coordinates of Solan area (H.P.) 30.9045º N, 77.0967º E (Fig. 3)

Preparation of plant material

Leaves of *Ficus palmata* were collected and dried in sunlight. After that, leaves were powdered with mechanical grinder and stored in airtight container. Samples were powdered separately. 1gm each of samples was taken for estimation of vitamins and minerals.

Estimation of tocopherol (Varley *et al*., 1991)

Principle

Tocopherol can be estimated using Emmeric- Engle reaction which based on the reduction of ferric to ferrous ions by tocopherols, which then forms a red colour 2.2’-dipyridyl-tocopherol and carotenes are first extracted

with xylene and the extraction read at 460 nm to measure carotenes. A correlation is made for these after adding ferric chloride and reading at 520 nm.

Reagents

1. Absolute alcohol
2. Xylene
3. 2,2'- dipyridyl
4. Ferric chloride solution
5. Standard solutions: Dissolved 10 mg/l of tocopherol in absolute alcohol. 91 mg of tocopherol is equivalent to 100 mg of tocopherol acetate.

Extraction

The sample was homogenized with water in a blender. Weighed accurately, 2.5 g of the homogenized sample into a conical flask. Add 50 ml of 0.1N H_2SO_4 slowly without shaking. Stoppered and allowed to stand overnight. The next day, contents of the flask were shaken vigorously and filtered through Whatman no.1 filter paper, discarding the initial 10-15 ml of filtrate. Aliquots of the filtrate were used for the estimation.

Procedure

Into 3 stoppered centrifuge tubes (test, standard and blank), pipetted out 1.5 ml of extract, 1.5 ml of standard, 1.5 ml of water respectively to the test and blank added 1.5 ml of ethanol and to the standard, added 1.5 ml of water. Added 1.5 ml of xylene to the test tubes, stoppered, mixed well and centrifuged. Transferred 1.0 ml of xylene layer into another stoppered tube taking care not to include any other ethanol or protein. Added 1.0 ml of 2,2'-dipyridyl reagents to each tube, stoppered and mixed. Pipetted out 1.5 ml of the mixture into colorimeter cuvettes and read the extinction of the test and standard against the blank, added 0.33 ml of ferric chloride solution. Mixed well and after 15 minutes read tests and standard against blank at 520 nm. The amount of Vitamin E can be calculated using the formula.

$$\text{Amount of Tocopherol} = \frac{\text{extinction at 520nm - extinction at 460} \times 0.29 \times 15}{\text{extinction at 520nm}}$$

Estimation of Ascorbic acid (Roe and Keuther, 1953)

Principle

Ascorbate is converted to dehydroascorbate by treatment with activated charcoal or bromine. Dehydroascorbic acid then reacts with 2,4 – dinitrophenylhydrazine to form osazones which dissolved in sulphuric acid to give an orange coloured solution whose absorbance can be measured spectrophotometrically at 540nm.

Reagents

1. 4% Trichloro acetic acid
2. 9 N sulphuric acid
3. 2% 2,4 – dinitrophenylhydrazine reagent(DNPH)
4. 10% Thiourea
5. 85% Sulphuric acid
6. Stock Standard solution: Dissolved 100mg of ascorbic acid in 100 ml of 4% TCA
7. Working Standard solution: Diluted 10 ml of stock solution to 100 ml with 4% TCA

Procedure

Ground 1g of the sample and homogenized in 4% TCA. Made up to 10 ml and centrifuged at 2000rpm for 10 minutes. The supernatant obtained was treated with a pinch of activated charcoal, shaken well and kept for 10 minutes. Centrifuged once again to remove the charcoal residue. Noted the volume of clear supernatant obtained 0.5 and 1 ml aliquots of this supernatant were taken for the assay. The assay volume was made up to 2.0 ml with 4% TCA 0.2 to 1.0 ml of working standard solution containing 20-100ug of ascorbate respectively was pipette out into clean dry test tubes and the volume was made up to 2.0 ml with 4% TCA. Added 0.5 ml of DNPH reagent to all the test tubes followed by 2 drops of 10% thiourea solution. Incubated at 37ºC for 3 hours. The ozone formed was dissolved in 2.5 ml of 85% sulphuric acid, in cold. Drop by drop with no appreciable rise in temperature. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. After incubation for 30 minutes at room temperature, the absorbance was measured spectrophotometrically at 540nm.

Nutrient analysis Na^+ and K^+

Instrument -Water bath, Hot plate

Procedure

1) Tri-acid mixture mix AR grade conc. HNO_3, H_2SO_4, $HClO_4$ of 10:4:1 ratio and cool.

2) Take 100mg of dried and processed plant sample into a 100 and 150 ml conical flask. Add 5 ml of conc. HNO_3

3) Keep a glass funnel on the flask, place it on a water bath and heat at 100 c for about 30 minutes

4) Shift the flask to a hot plate and heat at 180-200 C Measure temp. in a flask contain glycerol kept on the hot

plate.

5) Continue boiling until near to dryness but not drying completely. Cool and add 5 ml of tri-acid mixture (10:4:1).

6) Heat 180-200 C until the dense white fumes are evolved Continue digestion until the mixture is largely volatilized.

7) If the content is still brown cool a little and add 3-4 ml of tri acid mixture and continue as the digestion described above.

8) This is rare as there is still sufficient $HClO_4$ to the charged material.

9) Remove the flask when only moist clear and while contents are left. The entire quantity of $HClO_4$ has volatilized by this stage. Cool and add about 50 ml distilled water.

10) Filter into 100 ml flask giving washing to moving the volume of 100 ml. Use filtrate for analysis.

RESULTS

Comparative analysis of Sodium and Potassium ion, vitamin C and vitamin Ein healthy leaf and diseased of *Ficus palmata*

In the present study, the evaluation of nutrients is estimated and plant have maximum amount of potassium (33.33±10.929) and minimum amount of Sodium (1.333±0.333) in diseased leaf. The report is available and this shows that the amount of potassium is much higher in *Ficus palmata* as compared to sodium.. In the present study, the evaluation of nutrients is estimated and plant have maximum amount of potassium (42.00±2.081) and minimum amount of Sodium (1.333±0.333) in healthy leaf.

The present study showed that amount of sodium is equal in both leaf and potassium is greater in healthy leaf as compare to diseased leaf of plant. Tocopherol helps in antioxidant activity of plant greater amount has efficient antioxidant activity. In the present study estimation of vitamin c was done and the amount of tocopherol is higher in healthy leaf (2.0915±0.4188) and in diseased leaf (1.786±0.1383). This study reveals that healthy leaf has good antioxidant activity then diseased leaf. Norra (2011) reported that *Ficus deltoidea* showed antioxidant activity. The maximum antioxidant activity showed by tocopherol (97.67 ± 0.05) as compare to ascorbic acid (94.80 ±0.64).

In present study estimation of ascorbic acid was done and the amount of vitamin C is greater in diseased leaf as compare to healthy leaf. The amount of vitamin C in Diseased leaf (0.447±0.0386) and Healthy leaf (0.406±0.0108). Several studies show that the amount of ascorbic acid in leaf is lesser then the fruit or parts of plant. The present study on profiling of biotic stress effects on content of ascorbic acid, tocopherol and nutrients like sodium and potassium of *Ficus palmata* from Solan district Himachal Pradesh. Results of the study revealed that the plant extract has significant amount of Vitamin C. The other findings reported that healthy leaves have greater amount of potassium(K^+) (42.00±2.08167) and sodium ion (Na^+) (1.333±0.333) as compared to diseased leaf of *F. palmata*. The present study suggested that amount of tocopherol in healthy leaf is in more quantity as compared to diseased leaf of *F. palmata*. (Table 1)

The greater the amount of tocopherol, more will be the antioxidant activity of plant and is helpful in pharmaceutical industry, healthcare and antibiotic production etc. Tocopherol content is estimated as (2.091±0.4188) and (1.7863 ± 0.138) in healthy and diseased leaves, respectively and ascorbic acid is estimated as (0.447±0.0386) and (0.406±0.0108) in healthy and diseased leaves, respectively. The presence of vitamins and nutrients in plants helps in better antioxidant activity and this aided to the scavenging of reactive oxygen species (ROS). (Table 1)

Table 1: Comparative analysis of amount of Sodium and Potassium ion, vitamin C and vitamin E in Diseased and healthy leaf of *Ficus palmata*

S.No.	Parameters	Healthy leaf	Diseased leaf
1.	Na+	1.33±0.33	1.33±0.33
2.	K+	42.00±2.081	33.33±10.92
3.	Vitamin C	0.406±0.0108	0.44±0.038
4.	Vitamin E	2.091±0.418	1.78±0.138

Figure 1: Ficus palmata

Figure 2: Diseased leaf of Ficus palmata

Figure 3: Sampling site for collection of plant sample

DISCUSSIONS

The amount of Potassium 208.67 ± 7.09 and amount of Sodium 17.33 ± 2.08 (Hegazy *et al.*, 2013). The report was available showed comparison of nutrients in different medicinal plant and Na^+ was found in minor level as compare to K^+ (Rajurkar and Damame, 1998). Abolaji*et al*. (2007) reported that amount of sodium increased when fruit extract of *Parinari polyandra* level enhanced in mg/kg and level of potassium declined when fruit extract level of plant enhanced. . The amount of vitamin Cwas found in *Ficus palmata* (2.93 ± 0.18) in the fruit (Saklani, Chandra and Mishra, 2011). Ghazi*et al*. (2012) reported that content of ascorbic acid in *Ficus carica* 22.42±0.01 mg ascorbic acid/100 g and content of tocopherol found up to 1.9±0.57 mg tocopherol/100

mg. Doymaz (2005) reported that Vitamin C is abundant in figs.

ACKNOWLEDGEMENTS

I considerate privilege to work under the guidance of Dr Sujata Bhattacharya. Her continued encouragement and fatherly attitude have always helped me to keep going and to overcome the critical situations during my research work. I express my gratitude to her for her commitment and help which has greatly helped me in pursuing this work.

I am extremely grateful to Prof. Sunil Puri, Head of School, Shoolini University, Solan (HP) and Prof. Deepak Pathania, Dean, Faculty of Basic Sciences, Shoolini University, Solan (HP) for providing all the privileges during my research work.

No external funds are used for this research.

REFERENCES

Abolaji, A. O., Adebayo, H. A., & Odesanmi, O. S. (2007). Effects of ethanolic fruit extract of *Parinari polyandra* (Rosaceae) on serum lipid profile and some electrolytes in pregnant rabbits. *Research Journal of Medicinal Plant*, ***1*(4)**, 121-127.

Agarwal, S., & Grover, A. (2006). Molecular biology, biotechnology and genomics of flooding-associated low O_2 stress response in plants. *Critical Reviews in Plant Sciences*, **25(1)**, 1-21.

Akinmoladun, A. C., Ibukun, E. O., Afor, E., Obuotor, E. M., & Farombi, E. O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum. Scientific Research and Essays*, **2(5**), 163-166.

Bacon, C. W., & Yates, I. E. (2006). Endophytic root colonization by *Fusarium* species: histology, plant interactions, and toxicity. In *Microbial root endophytes* (pp. 133-152). Springer, Berlin, Heidelberg.

Bailey-Serres J, Voesenek LA (2008) Flooding stress: acclimations and genetic diversity. Annu. Rev. *Plant Biol*.; **59**, 313-339.

Bartels, D., & Sunkar, R. (2005). Drought and salt tolerance in plants. *Critical reviews in plant sciences*, **24(1)**, 23-58.

Bohnert, H. J., Nelson, D. E., & Jensen, R. G. (1995). Adaptations to environmental stresses. *The plant cell*, **7(7)**, 1099.

Carr, A. C., & Frei, B. (1999). Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *The American journal of clinical nutrition*, **69(6)**, 1086-1107.

Chinnusamy, V., & Zhu, J. K. (2009). Epigenetic regulation of stress responses in plants. *Current opinion in plant biology*, **12(2)**, 133-139.

Dingman, S. L. (2002). Water in soils: infiltration and redistribution. *Physical hydrology*, 575.

Doymaz, I. (2005). Sun drying of figs: an experimental study. *Journal of Food Engineering*, **71(4)**, 403-407.

Flowers, T. J. (1983). Sodium versus potassium: substitution and compartmentation. *Encyclopedia of plant physiology*, **15**, 651-681.

Frei, B., Stocker, R., England, L., & Ames, B. N. (1990). Ascorbate: the most effective antioxidant in human blood plasma. In *Antioxidants in therapy and preventive medicine* (pp. 155-163). Springer, Boston, MA.

Ghazi, F., Rahmat, A., Yassin, Z., Ramli, N. S., & Buslima, N. A. (2012). Determination of total polyphenols and nutritional composition of two different types of *Ficus carica* leaves cultivated in Saudi Arabia. *Pakistan Journal of Nutrition*, **11(11),** 1061.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.*, **43**, 205-227.

Glenn, E. P., Brown, J. J., & Blumwald, E. (1999). Salt tolerance and crop potential of halophytes. *Critical reviews in plant sciences*, **18(2)**, 227-255.

Greenway, H., & Munns, R. (1980). Mechanisms of salt tolerance in nonhalophytes. *Annual review of plant physiology*, **31(1)**, 149-190.

Halliwell, B. (1996). Commentary: vitamin C: antioxidant or pro-oxidant in vivo *Free radical research*, **25(5)**, 439-454.

Harrison, R. D. (2005). Figs and the diversity of tropical rainforests. *Bioscience*, **55(12)**, 1053-1064.

Hegazy, A. K., Al-Rowaily, S. L., Faisal, M., Alatar, A. A., El-Bana, M. I., & Assaeed, A. M. (2013). Nutritive

value and antioxidant activity of some edible wild fruits in the Middle East. *Journal of Medicinal Plants Research*, **7(15)**, 938-946.

Hoffman, P. C., Combs, D. K., & Casler, M. D. (1998). Performance of lactating dairy cows fed alfalfa silage or perennial ryegrass silage. *Journal of Dairy Science*, **81(1)**, 162-168.

Kadam, A. S., & Wadje, S. S. (2011). Role of potassium humate on growth and yield of soybean and black gram. *International J. Pharama Biol. Sci*, **1**, 243-246.

Keay, R. W. J. (1958). *Flora of west tropical Africa*. Crown Agents for Oversea Governments and Administrations.

Lansky, E. P., Paavilainen, H. M., Pawlus, A. D., & Newman, R. A. (2008). *Ficus* spp.(fig): Ethnobotany and potential as anticancer and anti-inflammatory agents. *Journal of Ethnopharmacology*, **119(2),** 195-213

Liu, B., Ren, J., Zhang, Y., An, J., Chen, M., Chen, H., & Ren, H. (2015). A new grafted rootstock against root-knot nematode for cucumber, melon, and watermelon. *Agronomy for sustainable development*, **35(1)**, 251-259..

Munné-Bosch, S., & Alegre, L. (2002). The function of tocopherols and tocotrienols in plants. *Critical Reviews in Plant Sciences*, **21(1)**, 31-57.

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, **59**, 651-681.

Norra, I. (2011). Free radical scavenging activity and phenolic content of *Ficus deltoidea* accessions MFD4 and MFD6 leaves. *Journal of Tropical Agriculture and Food Science*, ***39*(1)**, 85-92.

Pao, M. L., & Warner, A. J. (1993). Library Trends **41 (4)** 1993: The Depreciation of Knowledge.

Prajapati, N. D., & Prajapati, T. (2002). Sustainable cultivation of medicinal plants; Multi tier agriculture system-A new concept.

Prior, R. L., & Cao, G. (2000). Antioxidant phytochemicals in fruits and vegetables: diet and health implications. *HortScience*, ***35*(4)**, 588-592.

Rajurkar, N. S., & Damame, M. M. (1998). Mineral content of medicinal plants used in the treatment of diseases resulting from urinary tract disorders. *Applied radiation and isotopes*, **49(7)**, 773-776.

Rao, R. V., Poksay, K. S., Castro-Obregon, S., Schilling, B., Row, R. H., Del Rio, G & Bredesen, D. E. (2004). Molecular components of a cell death pathway activated by endoplasmic reticulum stress. *Journal of Biological Chemistry*, **279(1)**, 177-187.

Ray, W. A., Meredith, S., Thapa, P. B., Meador, K. G., Hall, K., & Murray, K. T. (2001). Antipsychotics and the risk of sudden cardiac death. *Archives of General Psychiatry*, **58(12)**, 1161-1167.

Rodriguez, R. J., Henson, J., Van Volkenburgh, E., Hoy, M., Wright, L., Beckwith, F. & Redman, R. S. (2008). Stress tolerance in plants via habitat-adapted symbiosis. *The ISME journal*, **2(4)**, 404.

Saklani, S., Chandra, S., & Mishra, A. P. (2011). Evaluation of antioxidant activity, quantitative estimation of phenols, anthocynins and flavonoids of wild edible fruits of Garhwal Himalaya. *Journal of Pharmacy Research*, ***4*(11)**, 4083-4086.

Shimwell, D. W. (1971). The description and classification of vegetation sedgwick and jackson. *London*, **322**.

Shinwari, Z. K., Pehman, M., Watanabe, T., & Yoshikawa, T. (1996). A pictorial guide to medicinal plants of Pakistan Kohat University of Science and Technology.

Srivastava, J. P., Lambert, J., & Vietmeyer, N. (1996). *Medicinal plants: An expanding role in development*. The World Bank.

Strobel, G. A. (2002). Book review: "Mechanisms of Resistance to Plant Diseases, AJ Slusarenko, RSS Fraser, LC van Loon (Eds.), Dordrecht: Kluwer Academic Publishers (2001), 620 pp." *Plant Science*, **1(163),** 177-178.

Taylor, R. S., Manandhar, N. P., & Towers, G. H. N. (1995). Screening of selected medicinal plants of Nepal for antimicrobial activities. *Journal of Ethnopharmacology*, **46(3)**, 153-159.

Thomas, D. R., Ashmen, W., Morley, J. E., & Evans, W. J. (2000). Nutritional management in long-term care: development of a clinical guideline. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, ***55*(12)**, M725-M734.

Van Brunt, J. M., & Sultenfuss, J. H. (1998). Better crops with plant food. *Potassium: Functions of potassium*, **82(3)**, 4-5.

Ward, J. P., & Robertson, T. P. (1995). The role of the endothelium in hypoxic pulmonary vasoconstriction. *Experimental Physiology: Translation and Integration*, **80(5)**, 793-801.