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Increase in Yield and Immunity in Bam O™ Treated Papaya Plants in India

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ABSTRACT

Background: *Carica papaya* L. (family Caricaceae) is one of the popular fruits in world. As with many tropical and subtropical crops, papaya is host to various pests and pathogens. More than 700 papaya plants were infected by unknown virus. The incidence of plants infected with leaf curl disease was 50-75%, but the infection was very severe. A typical curling and thickening were observed. The infection spread by air and rainwater to nearby papaya farms.

Methods: This study reports the effect of BamO formulation on the biotic stress of the Papaya plant. BamO was sprayed on the plants and its effect was observed. After six days of Bam O treatment, new healthy leaves were observed on the plants.

Result: The yellow and old leaves were dropped from the plants. After 2 months of the treatment BamO, the fruits were elongated and having similar shapes without uneven bumps. The green fruits without any dark or light spots were observed in BamO treated plants. Priming effect was observed by GCMS analysis, the 3 methylsalicylic acid increased quantity was observed in 7th day and 30th day samples of BamO treated plants. The BamO treatment not only increased the immunity of the plant but found effective in increasing yield of the papaya plants. In BamO treated papaya plants, the total yield obtained was 92.10 tonnes per 444 plants.

Key words : BamO, *Carica papaya* L., GCMS, Priming.

INTRODUCTION

Carica papaya L. (family Caricaceae) is one of the major fruits produced worldwide (FAO, 2021). India produces an incredible 4.2 million metric tonnes, accounting for 35% of the world's production. The productivity of papaya increased to 5.89 metric million tonnes through adoption of high-yielding cultivars and practicing improved agro-techniques (Kaur and Kaur, 2017). Papaya fruit contains high amount of vitamin A, B_1 and B_2 as well as important proteolytic enzyme papain, which helps in digesting protein rich food, with several pharmaceutical applications (Santana *et al*., 2019; Teixeira *et al*., 2012; Vij and Prashar, 2015). There are number of incidences reported regarding loss, tragedy, poor quality and misery associated with agricultural crops that are threatened by various biotic and abiotic factors (Pandey *et al*., 2021). Many biotic factors include bacteria, fungi, viruses, virus-like organisms and nematodes, which infect the crops and retard the production yield (Kataria and Kumar, 2012) along with significant economic loss. Among these biotic factors, viruses create major constraints to agriculture production in India (Patil *et al*., 2018; Prabha and Modgil, 2018).

As with many tropical and subtropical crops, papaya is host to various pests and pathogens. Begomoviruses one of the principal genus of the family Geminiviridae encompass more than 320 diverse species prevailing in the tropical and subtropical vicinity of the old and new world, infects crops, ornamentals and weed plants; they are chiefly transmitted by the vector white-fly (*Bemisia tabaci*) (de Barro *et al*., 2011; Krishna Reddy *et al*., 2010; Marwal *et al*., 2017; Prajapat *et al*., 2014). In India, the first study of papaya leaf curl disease caused by the begomovirus Papaya leaf curl virus (PaLCV) was reported in 1998 (Malathi *et al*., 2017; Saxena *et al*., 1998) (Nehra *et al*., 2019).

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MATERIALS AND METHODS

Papaya plant leaves collection

The papaya plants were obtained from a village near Partur, Dist. Jalna, India (19.8410°N 75.8864°E). Atmosphere of the place for the month of Sept. 2020 was found rainy and moist. The average daily temperature ranges 20° to 27°C. A one-hectare infected plant farm was selected for the study. The leaves of the plants were collected from the study area.

Papaya plant external symptoms

The papaya plants were found with severe viral or fungal infections. A typical curling and thickening were observed

(Fig 1). These included chlorotic spots (Fig 1), ringspots and line pattern (Fig 1), leaf distortion, mosaic, dieback and curling. In plants showing distortion symptoms, greasy streaks and grey spotting were usually found on stems. Whether in the field, infected plants initially developed vein banding symptoms, accompanied in some cultivars by mosaic, distortion and occasional stunting. Ringspot symptoms sometimes appeared on the fruits, but virus could not be recovered from such fruits. All the symptoms suggested an infection of the ringspot virus in the papaya plants (Allan, 1980).

Application of BamO on papaya plants

BamO solution was prepared using 4.5 kg BamO powder mixed with 9 litres of water. The stock solution was used to prepare dilutions of the BamO working solutions. BamO formulation was diluted to 1:250, 1:500 and 1:1000. More than 400 plants were used for treatment. The BamO was sprayed on plants as per schedule given in Table 1.

Analysis of secondary metabolites from leaves

Metabolites extraction

The leaves of the BamO Sprayed papaya plants and Untreated plants were collected from the site. The metabolites of treated samples were extracted using a hot methanolic extraction method. Hot methanolic extraction: 10 mL methanol:water (1:1) was mixed with 1 gram of crushed seeds/seedlings and kept at 70°C for 15 min. Then, the mixture was incubated for room temperature and used for the preparation of esters.

Methyl ester preparation: 250 µg or 50 µL of lipid samples were added to 1 mL of 1% methanolic NaOH (freshly prepared). The samples were heated at 55°C for 15 min. In hot tubes, 2 ml of 5% methanolic HCl (freshly prepared) was added and the mixture was again heated for 15 min at 55°C. The fatty acid methyl esters (FAME) were eluted by adding 1 mL of hexane to the above mixture. FAME were then stored at 8-10°C.

GCMS

The methyl esters obtained from the samples (as described in previous subsection) were used for GCMS. GCMS analysis (Agilent 5975 C gas chromatography system) was performed following the users' guide to calibrate method with FAME standards, available on Fiehn GC-MS Metabolomics library-2008 (Agilent Chem Station, Agilent Technologies Inc., Wilmington, USA), with slight modifications, using retention time locking method. HP-5MS capillary column (30 m length, 10 m Duraguard, 0.25 mm diameter; narrow bore and 0.25 µm film) manufactured by Agilent JandW GC columns, USA, was used for the analyses. Following oven temperature program was maintained: 60°C (1 m), followed by 325°C at 10°C/min as final hold for 10 min before cool-down. Run time was 37.5 min. Injection temperature was set at 250°C, MSD transfer line at 290°C and ion source at 230°C. Helium was used as carrier gas (constant flow rate of 0.723 mL/min; carrier linear velocity 31.141 cm/s). Sample (1 µL) was injected onto the column via split mode (split ratio was 1:5). Chromatograms were analysed using Automated Mass Spectral Deconvolution and Identification System (AMDIS). Metabolite identification was performed by comparing the retention times (Rt), retention indices (RI) and mass spectral fragmentation pattern of compounds using the references present in Agilent Fiehn Metabolomics library. Many of the metabolites were also identified by comparing chromatographic and spectral properties with that of standard compounds.

Analysis of the growth and yield of papaya fruit

Leaf colour, size, shape and number were observed from BamO treated and untreated control plants. The fruits size, shape, weight and colour were recorded. The plant height and size were recorded.

RESULTS AND DISCUSSION

Effect of BamO on diseased Papaya plants

All papaya plants were grouped into two different groups – BamO treated and untreated control plants. The BamO treated plants initially had same yellow leaves, spots on stem and very narrow leaves (Fig 1). The untreated plants had the spots on the stem, leaves and fruits. The new leaves also got infected and the curling effect was observed (Fig 1A). The new fruits were unhealthy and damaged. Fruits had uneven bumps; leaves presented a bright yellow mosaic pattern and new leaves were small having curled shape and plant growth was stunted. young fruit withered and dropped from plants (Fig 1B). The leaves were curled and brown; trunks had raised lesions; fruit had sunken circular lesions (Fig 1C). After six days of BamO treatment, new healthy leaves were observed on the plants. The yellow and old leaves had dropped and the new young fruit had healthy shapes (Fig 2A to C).

After the 12th day of BamO treatment, it was observed that the yellow leaves had dropped. (Fig 3A and 3B).The plants were growing and the number of new fruits were increasing on BamO treated plants (Fig 3).

On 27th day of BamO treatment new leaf groups are found. Green healthy BamO treated plants were observed on 27th day of BamO treatment. Fruit size and numbers were normalized. The dark/faint spots were reduced from the leaves. The healthy green leaves and stems were

Table 1: Application of Bam-O on plants.

Days	Treatment
Day 0	Bam O 1:250 sprayed on all 400 plants
Day 5th	Bam O 1:250 sprayed on all 400 plants
Day 7th	Bam O 1:250 given by drenching
Day14th	Bam O 1:250 given by drenching
Day 21st	Bam O 1:500 given by drenching
Day 22nd	Bam O 1:250 sprayed on leaves of all plants
Day 29th	Bam O 1:1000 given by drenching

After 2 months of the treatment BamO, the production of the papaya was observed. The fruits were elongated and had similar shapes without uneven bumps (Fig 4 C and D). Green fruits without any dark or light spots were observed in BamO treated plants (Fig 4B and C). In untreated papaya plants, the fruits were very low in number, the leaves were few and yellow. The fruit size was very small to moderate (Fig 5A and B).

The BamO treated plants were healthy and had more than 20 fruits per plant. The leaves were wide, green and spotless. The yellow leaves dropped from the plants. The fruits were spotless and each fruit was 5 to 8 kg in weight (Fig 4C and D).

Metabolites detection by GCMS

Plant leaves were crushed in methanol and kept at 4°C for 14 hours. After 14 hours, the esters were prepared and analysed by GCMS. Benzoic acid was induced after one week (Fig 6) After one week of the first spray of BamO on papaya plants, data showed the following: presence of 3 methylsalicylic acid; 6, bromohexanoic acid; benzoic acid; 1, 2-cinnolinedicarboxylic acid; 2,6 dihydroxybenzoic acid; 3,4 dimethylbenzoic acid. After one month of the first spray, data showed that 2, 6 dihydroxybenzoic acid increased compared to the the seventh day sample. New 3,4 dihydroxymandelic acid, 3-hydroxypropionic acid and trans 2-4 Dimethoxy cinnamic acid were found in only one month leafsamples. The 3 methylsalicylic acid expression was increased in the one-month sample (Fig 6). This clearly indicates the priming effect of the BamO on papaya plant to increase the natural immunity of the plant against the biotic stress.

Yield of the Papaya fruits

The yield of the papaya fruits was calculated from three different groups. The untreated diseased plant yield was 12 tonnes per 200 plants. The average weight of the fruit was 2.6±0.5 kg and 7-30 fruits were observed per plant (Table 2). In the second group, normal healthy untreated 180 plants , the yield was 19 tonnes with an average fruit weight 3.57±0.6 kg (Table 2). The BamO treatment not only increased the immunity of the plant but was effective in increasing yield of the papaya plants. The average fruit weight was 6.4±1.0 kg and more than 23-39 fruits per plant . The total yield was

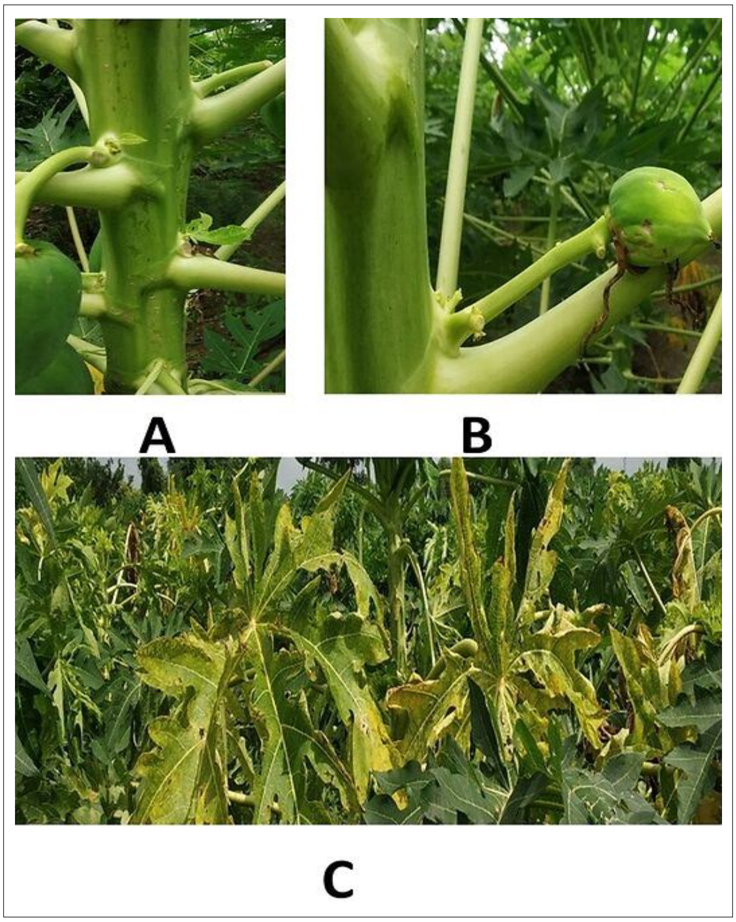

Fig 1: *Carica papaya* diseased plants.
A); Spots on the stem of the papaya plant, B); Damaged fruit of the infected plant, C); Leaf distortion, mosaic, dieback and curling of leaves on infected plant.

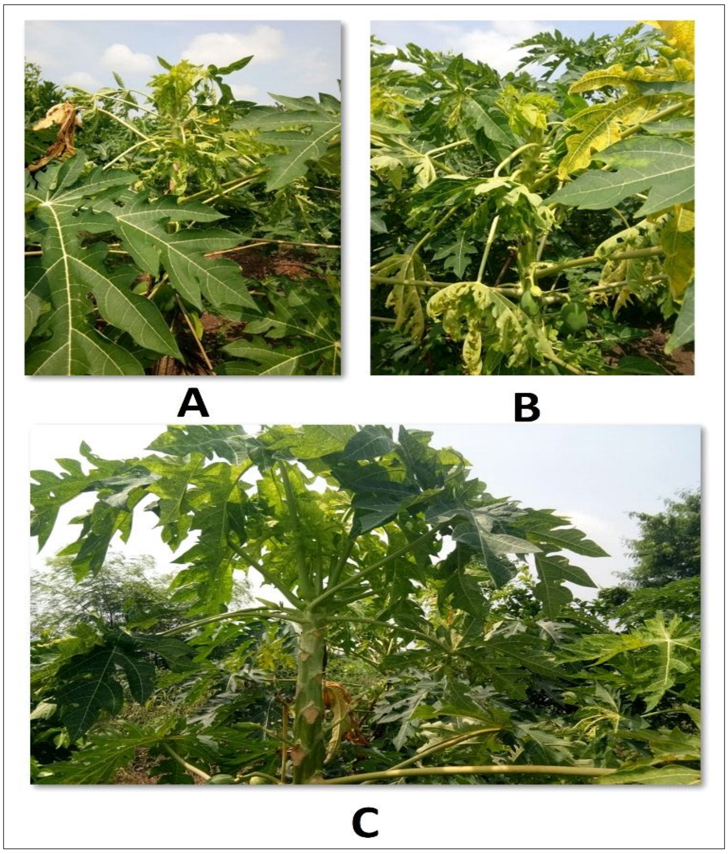

Fig 2: BamO sprayed *Carica papaya* plant after 6 days of first spray. A) After six days of Bam O treatment , new healthy leaves were observed on the plants; B) The new young fruits with healthy shapes; C) The yellow and old leaves had dropped from the plants.

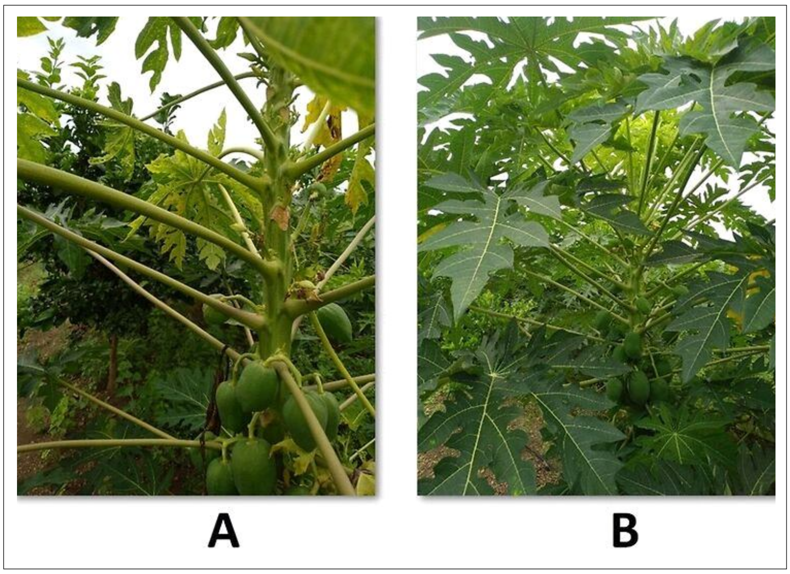

Fig 3: BamO sprayed Carica papaya plant after 12 days of first spray. A); After 12 days of BamO treatment, new healthy leaves were observed on the plants. The new young fruits had healthy shapes; B); The new green leaves on the BamO treated plants.

Table 2: Papaya fruit yield from BamO treated and untreated plants.

Papaya fruits	Average Weight/fruit	Number of fruits obtained after five harvest cycles per plant min-max	Number of plants	Total yield in tonnes
Untreated Virus infected plant	2.6±0.5 kg	7-30	200 plants	12 Tonnes
Untreated normal healthy plants**	3.57±0.6 kg	12-31	180 plants	19 Tonnes
BamO treated plants	6.4±1.0 kg	23-39	444 plants	92.10 Tonnes

** These plants were classified as a normal from their appearance.

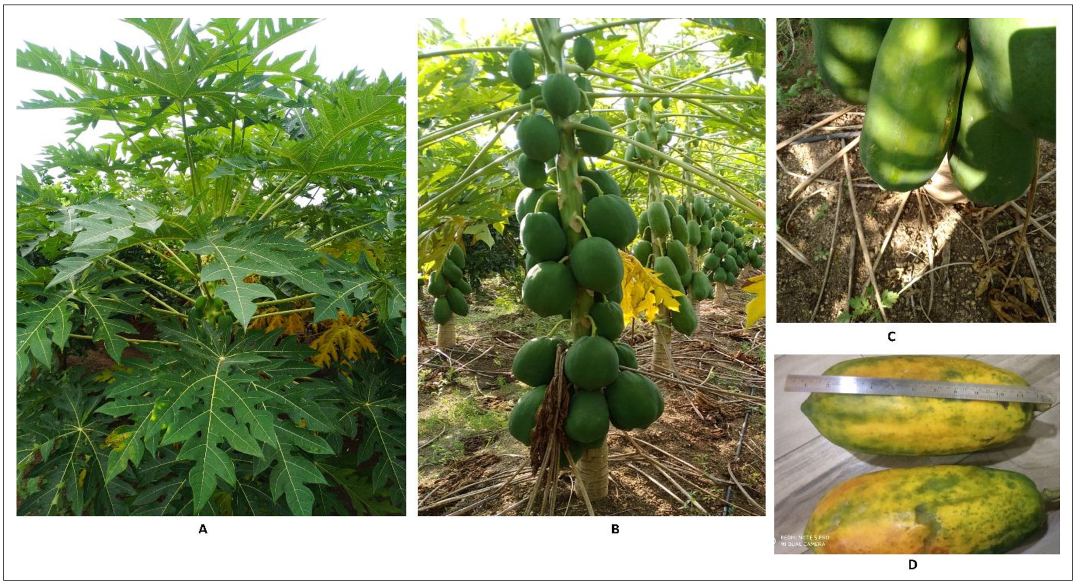

Fig 4: BamO sprayed *Carica papaya* plant after 12 days of first spray.

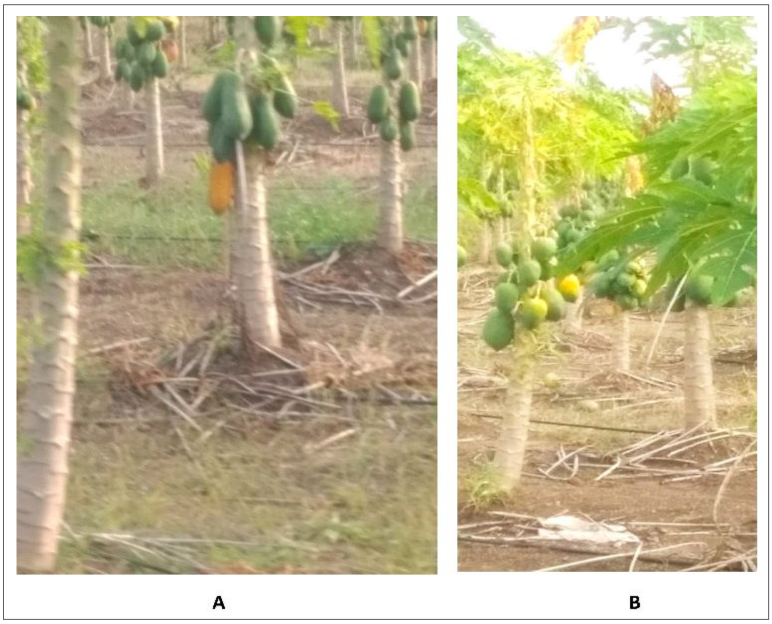

Fig 5: Diseased Carica papaya plants untreated control.

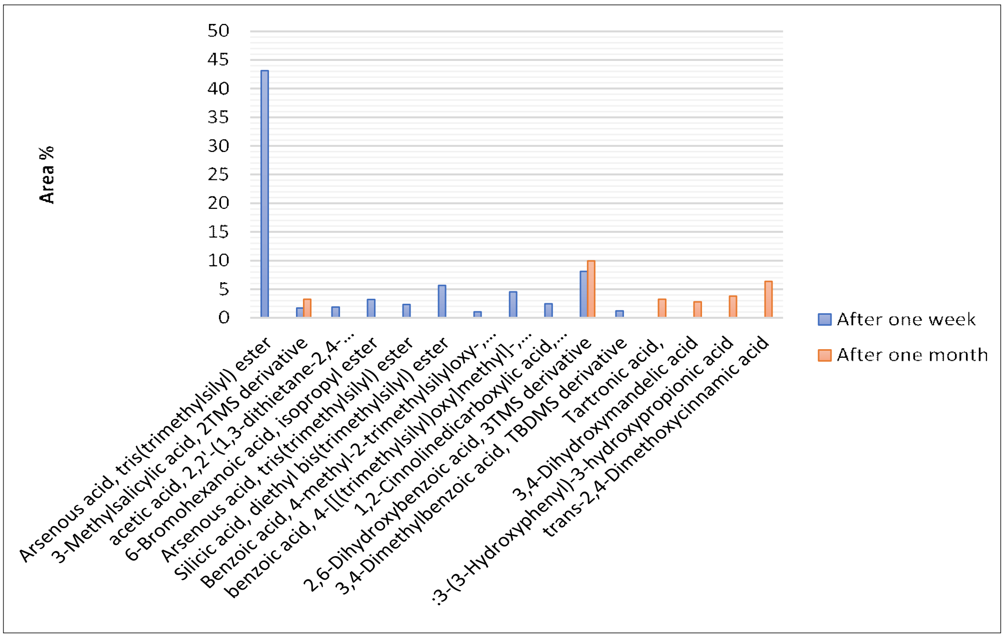

Fig 6: The major metabolites induced in the BamO treated papaya plants .

Where- After one week- BamO treated plant leaves after one week of treatment, After one month- BamO treated plant leaves after one month.

92.10 tonnes per 444 plants (Table 2). The increased yield in BamO treated plants clearly indicates that the priming effect boosts the productivity of the plants.

CONCLUSION

In summary, this study confirms the priming effect of BamO on diseased papaya plants. The priming effect was observed by GCMS analysis and the elevated 3 methylsalicylic acid was observed in 7^{th} day and 30^{th} day samples of BamO treated plants. This clearly indicates the priming effect of BamO on papaya plant to increase the natural immunity of the plant against the biotic stress. The BamO treatment not only increased the immunity of the plant, but was effective in increasing yield of the papaya plants. The increased yield in BamO treated plants clearly indicates the priming mechanism boosts plant productivity.

Conflict of interest: None.

REFERENCES

Allan, F.L. (1980). Transmission and properties of viruses isolated from *Carica papaya* in Nigeria. Journal of Horticultural Science. 55(2): 191-197. https://doi.org/10.1080/00221589.1980.11514922.

de Barro, P.J., Liu, S.S., Boykin, L.M. and Dinsdale, A.B. (2011). Bemisia tabaci/ : A statement of species status. Annual Review of Entomology. 56(1): 1-19. https://doi.org/10.1146/annurev-ento-112408-085504.

FAO. (2021). The State of Food and Agriculture 2021. Making agrifood systems more resilient to shocks and stresses. Rome, FAO. https://doi.org/10.4060/cb4476en.

Kataria, R. and Kumar, D. (2012). Occurrence and infestation level of sucking pests: Aphids on various host plants in agricultural fields of Vadodara, Gujarat (India). Int J. Sci Res Publication. 2: 1-6.

Kaur, K. and Kaur, A. (2017). Papaya performance under various growing conditions cv. Red Lady 786. Agricultural Science Digest - A Research Journal. 37(4): 290-293. https://doi.org/10.18805/ag.D-4681

Krishna, R.M., Venkataravanappa, V., Madhuvanthi, B. and Jalali, S. (2010). Molecular characterization of begomoviruses associated with papaya leaf curl disease in india. Acta Horticulturae. 851: 465-472. https://doi.org/10.17660/Acta Hortic.2010.851.72.

Malathi, V.G., Renukadevi, P., Chakraborty, S., Biswas, K.K., Roy, A., Sivalingam, P.N., Venkataravanappa, V. and Mandal, B. (2017). Begomoviruses and Their Satellites Occurring in India: Distribution, Diversity and Pathogenesis. In: A Century of Plant Virology in India (pp. 75-177). Springer Singapore. https://doi.org/10.1007/978-981-10-5672-7-5.

Marwal, A., Mishra, M., Sekhsaria, C. and Gaur, R.K. (2017). Computational Analysis and Predicting Ligand Binding Site in the Rose leaf curl virus and Its Betasatellite Proteins: A Step Forward for Antiviral Agent Designing. In: Begomoviruses: Occurrence and Management in Asia and Africa (pp. 157-168). Springer Singapore. https://doi.org/10.1007/978-981-10-5984-1-9.

Nehra, C., Marwal, A., Verma, R.K., Mishra, M., Sharma, P. and Gaur, R.K. (2019). Papaya yellow leaf curl virus/ : A newly identified begomovirus infecting *Carica papaya* L. from the Indian Subcontinent. The Journal of Horticultural Science and Biotechnology. 94(4): 475-480. https://doi.org/10.1080/14620316.2019.1570827.

Pandey, V., Srivastava, A. and Gaur, R.K. (2021). Begomovirus: A curse for the agricultural crops. Archives of Phytopathology and Plant Protection. 54(15-16): 949-978. https://doi.org/10.1080/03235408.2020.1868909.

Patil, R.V., Patil, V.R. and Pujari, C.V. (2018). Effect of maturity stages on shelf life of papaya (*Carica papaya* L.). Asian Journal of Dairy and Food Research, https://doi.org/10.18805/ajdfr.DR-1340

Prabha, A. and Modgil, R. (2018). Comparative nutritional quality evaluation of different cultivars of Papaya. Asian Journal of Dairy and Food Research. 37(2): 158-161. https://doi.org/10.18805/ajdfr.DR-1299

Prajapat, R., Marwal, A. and Gaur, R.K. (2014). Begomovirus associated with alternative host weeds: A critical appraisal. Archives of Phytopathology and Plant Protection. 47(2): 157-170. https://doi.org/10.1080/03235408.2013.805497.

Santana, L.F., Inada, A.C., Espirito, S.B.L.S., Filiú, W.F.O.D., Pott, A., Alves, F.M., Guimarães, R.D.C.A., Freitas, K.D.C. and Hiane, P.A. (2019). Nutraceutical potential of *Carica papaya* in metabolic syndrome. Nutrients. 11(7): 1608. https://doi.org/10.3390/nu11071608.

Saxena, S., Hallan, V., Singh, B.P. and Sane, P.V. (1998). Nucleotide sequence and intergeminiviral homologies of the DNA-A of Papaya leaf curl geminivirus from India. IUBMB Life. 45(1): 101-113. https://doi.org/10.1080/15216549800202472.

Teixeira, L.G., Lages, P.C., Jascolka, T.L., Aguilar, E.C., Soares, F.L.P., Pereira, S.S., Beltrão, N.R.M. *et al*. (2012). White tea (*Camellia sinensis*) extract reduces oxidative stress and triacylglycerols in obese mice. Food Science and Technology. 32(4): 733-741. https://doi.org/10.1590/S0101-20612012005000099.

Vij, T. and Prashar, Y. (2015). A review on medicinal properties of *Carica papaya* Linn. Asian Pacific Journal of Tropical Disease. 5(1): 1-6. https://doi.org/10.1016/S2222-1808(14)60617-4.