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Citrus Decline

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A B S T R A C T

Citrus, a collective generic term, comprising a number of species and varieties of fruits, popular at global level for their characteristics flavor and attractive range of colours, citrus fruits are good source of vitamin-C. It is originated from southern slopes of Himalayas, entire north-eastern region of India and adjacent China. Citrus consists of number of species but 8 species are generally cultivated for edible purpose. Citrus is spread throughout the tropics and subtropics, roughly 40°N and 40°S latitudes. The major region subtropical latitude of northern hemisphere is North America, Mediterranean, East Asia including India and southern hemisphere is South America, South Africa, Australia. The total global area on citrus accounts to 9.68 million hectares producing 135.76 million tonnes. India covers 953.4 thousand ha and producing 11655 thousand tonnes of citrus. Northeastern states are accounting 147 thousand hectares citrus groves producing 868 ('000 tonnes). Assam covers 29 thousand hectares citrus area and producing 309 thousand tonnes. India has 4th position at global level in citrus production. It is 3rd most important fruit crop in India. Citrus growing regions of the world are threatened by a complex problem causing the decline of citrus orchards, citrus decline is serious setback in all over the world. Citrus decline (complex problem) gradually decline of production, productivity of orchards, finally orchards become unproductive. Major causes of citrus decline – Abiotic and biotic, such as Abiotic causes consist- soil and nutrient related problems, moisture content, physiological disorders. Biotic causes are rootstock, phanerogamic parasites, intercrops, insects-pest, nematodes and diseases (fungal, bacterial and viral). Major causes of citrus decline in Northeastern India: 1. Citrus trunk borer; 2. Occurrence of greening disease exists in north eastern citrus growing region (Ghosh *et al.*, 1982). Greening and tristeza are present in Khasi mandarin, tristeza is also present in Kagzi lime and Assam lemon in the NE of India by Bhagabati *et al.*, (1989). Das *et al.*, (2007) also reported greening disease in NE states of India through PCR technique. This is the main cause of citrus decline in NE states; 3. High Incidence of CTV (Singh *et al.*, 2017) in NE states of India; 4. Phytophthora rot and 5. Overall neglect of grove.

Introduction

Citrus, a collective generic term, comprising a number of species and varieties of fruits, popular at global level for their characteristics flavor and attractive range of colours. Its attractive evergreen foliage and fragrance are added aesthetic value of citrus trees, citrus fruits are good source of vitamin-C. It is originated from southern slopes of Himalayas, entire north-eastern region of India and adjacent China. Citrus consists of number of

species but 8 species are generally cultivated for edible purpose. Citrus is spread throughout the tropics and subtropics, roughly 40°N and 40°S latitudes. The major region subtropical latitude of northern hemisphere is North America, Mediterranean, East Asia including India and southern hemisphere is South America, South Africa, Australia. The total global area on citrus accounts to 9.68 million hectares producing 135.76 million tonnes. India covers 953.4 thousand ha and producing 11655 thousand tonnes of citrus. Northeastern states are accounting 147 thousand hectares citrus groves producing 868 ('000 tonnes). Assam covers 29 thousand hectares citrus area and producing 309 thousand tonnes.

India has 4th position at global level in citrus production. It is 3rd most important fruit crop in India. Citrus growing regions of the world are threatened by a complex problem causing the decline of citrus orchards, citrus decline is serious setback in all over the world such as Nagpur Santra of central India, Mosambi Orange of W. India, Khasi Mandarin- N.E. India, Kinnow- N. India, Coorg Mandarin- S. India, Satsuma mandarin belts of Japan, orange growing tracts of Australia, Philippines, Indonesia, S. America, Argentina, Brazil, California and Florida.

Citrus decline (complex problem) gradually decline of production, productivity of orchards, finally orchards become unproductive. Seriousness of this complex problem observed in 19th century. First observance recorded in 18th century by Roghoji Bhonsale in the central province, in Assam first observed in the year 1888. The decline of sweet orange, mandarin and grapefruit varieties grafted on sour orange led to the loss of about 100 million. Seriousness was first recognized by Department of Agriculture, Bombay state in 1912, in Punjab in 1940 and became very severe in early 50s.

Research has done for resolving this problem in all over the India at various research stations as following. Around 1954, the ICAR, New Delhi launched an elaborate research project on citrus die back problem at 3 centers.

Gonikoppal (Coorg mandarin) in Karnataka,
Shrirampur (Sweet orange) in Maharashtra
IARI, New Delhi (plant physiology division)

In 1962 another research on die back was initiated at IARI, new Delhi, NRCC, Nagpur in Maharashtra, AICRP at research center in A.P (Tiruapati), Assam (Tinsukia), Maharashtra (Akola and Rahuri), Punjab (Ludhiana) and Tamilnadu (Periakulam), IIHR, Bangalore initiated pilot cum demonstration trials in early 70s, Byrnihat, Assam (now in Meghalaya) in 1933.

Factors responsible for citrus decline

The factors responsible for decline can be grouped as abiotic and biotic factors as following: 1.) Abiotic factors 2.) Biotic factors

Abiotic factors

Nutrient imbalance

Balance of the nutrients both in the soil and plants is essential for proper growth and development, malnutrition also responsible for citrus decline. Excess or deficiency both are harmful to plants such as due excess phosphorus crinkle rind in citrus fruits, excess zinc shows iron chlorosis and induces leaf burn, defoliation, twig dieback. In copper excess, plants become stunted, roots stubby dark coloured and show iron chlorosis. Zn deficiency shows little leaf, rosette leaves symptoms and due to copper deficiency dieback occurs, molybdenum deficiency causes yellow spots in citrus.

Soil related constraints

Unsuitable soil is another major factor for declining of an orchard, excessive free lime, excessive salts, defective drainage, presence of hard pan and low soil fertility are some of the soil properties found responsible for decline of citrus plantations. The best pH for citrus ranges from 5 to 6, in soil which is too acidic citrus roots don't grow well, nutrients are leached out or may even become toxic, (viz. copper, aluminum, iron), at a pH above 6 fixations of nutrients especially iron and zinc will take place and plants shows deficiency.

Physiological disorders

Fruit drop

Fruit drop occurs due to formation of abscission layer at the stem end, this is largely due to imbalance of certain hormones. Application of 2, 4-D @ 8-10 ppm and 2, 4, 5-T @ 20 ppm are effectively reduces fruit drop.

Splitting

It is common disorder of sweet orange, most of splitting originates from naval and stylar end of fruits. Splitting caused by fluctuating physiological factors such as soil moisture, temperature, relative humidity.

Granulation

Juice sacs become tough, enlarged nearly colourless, tasteless, juice vesicles become thicker, pectic substances increases, sugar, organic acids, carotenoids decrease whereas mineral constituents increase. Water get bounds in gels, the exact cause of granulation is unknown but some factors high soil moisture, high temperature during fruit development and high relative humidity also in influence this disorder. Spraying of 16 ppm 2,4-D on developing fruits may overcome granulation.

Biotic factors

Rootstock

Selection of suitable rootstock is very important in establishing of healthy orchard for higher productivity and longevity. The use of improper rootstocks causes incompatibility which is genetic or physiological incongeniality between stock and scion leads to decline of citrus orchards.

Intercrops

Selection of good intercrop influence production and income too, but undesirable intercrops adversely affect the trees through exhaustion of nutrients, deterioration of soil physical properties, harbouring insect-pests and diseases. Exhaustive crops like wheat, maize, sugarcane are unfavourable for citrus, potato, berseem, tobacco, tomato, chilies caused severe infestation of root knot nematodes.

Phanerogamic parasites

Loranthus feeds on the host leading dieback, death of infested twigs and dodder carry viruses, feed on hosts.

Fungi

Phytophthora diseases

Numerous fungi infects citrus plants belongs to genus Phytophthora, *viz. P. palmivora, P. citropthora, P. parasitica.* The fungi are soil inhabitant prolonged exposure of citrus trees to wet soil condition is more prone to their infection. Symptoms of gummosis dead areas are adhering to the trunk firmly, exudation of gum and brown staining of wood, subsequent

drying and vertical cracking of bark. Symptoms of foot rot less noticeable gum due to absorption of gum by soil water, fungus also causes seedling dumping off and leaf fall in citrus. Drenching of 0.2% foltaf thrice at monthly interval is effectively control foot rot and gummosis.

Powdery mildew

Powdery mildew (*Oidium tingitaninum*) is common disease in citrus groves.

It causes white patches or white powder on upper surface of the leaves, twigs leading to leaf drop and dieback. Sulfur fungicides are commonly used for controlling this like sulfex 0.3%.

Scab

It caused by *Elsinoefawcetti*, symptoms are corky lesions on leaves, twigs and fruits. Distortion of leaves, hardening of fruits premature fruit drop in case of sever infestation, sparay of Bavistin 0.05%, Foltaf 0.25%. Cleopatra, Sevage citrange, citrumelo and Box orange are free from scab infection.

Twig blight/wither tip

Drying of twigs from tip to downward and leaf defoliation, colour of blighted twigs become greyish white and studded with numerous dots of fungus *Colletotrichum gloeosporiodes*, various fungi *Diplodia natelensis, Fusarium solani, Botrytis cinerea, Sclerotinia sclerotiorum* were found to be associated with twig blight disease. Pruning of blighted twigs followed by 2 sprays of Bavistin 0.1%

Lichens

Under moist condition lichens are found on twigs and branches on citrus, weak bordeaux mixture or strong lime solution are useful for controlling lichens.

Algal spot

Cephaleuros mycoidea causes slightly raised spots on leaves, twigs, fruits in wet humid citrus belts.

Bacteria

Bacterial canker caused by *Xanthomonas compestris pv. citri* is an important disease of citrus throughout the world. The citrus species are susceptible to canker are lemons, lime, sour orange, sweet orange, grapefruit, trifoliate orange, satsuma mandarin. Calamondin, kumquat, tangerines are found to be resistant. Eradication is very effective for controlling of canker and spraying of 500 ppm streptomycin sulphate.

Viruses

Several virus diseases observed in citrus, in India fourteen viral diseases observed such Tristeza, Psorosis, Greening are the major viral diseases of citrus.

Quick decline or citrus tristeza disease

Quick decline is wide spread throughout the citrus growing regions of the world. Stem pitting and seedling yellows are the main symptoms. The infected trees show absence of new growth, defoliation, root decay, stunted growth, twig dieback leading to death of plants. Control measures are: Control of vectors (aphids) through insecticides such Monocrotophos, Use of resistant rootstocks-Jattikhatti, Rangapur lime, Cleopatra mandarin, Troyer citrange or Swingle citromelo**.** Cross protection techniques as a vaccination of young seedlings, CRISPR technology editing of genes, RNA Interference- post-transcriptional gene

silencing, RdRP (pathogen derived resistance), Coat proteins-p20 and p23, p20 and p25 (intercellular level). Heat shock proteins- HSP-61, HSP-65 alteration in vectors forgut to create problem in the binding of virus Biocontrol agents- *Aphelinus gossypii, Coelophera inaequalis, Pseudodorus clavatus*, Quarantine is the best management strategy.

Psorosis

Psorosis citrus disease complex found in most of citrus growing belts, psorosis A causes bark scaling of trunk and large branches, psorosis b- initial flow of gum followed by bark scaling.

Greening

Serious disease of citrus first reported by Fraser *et al.,* 1966, symptoms- leaf chlorosis resembling Zn deficiency, short twigs narrow and upright yellow leaves, premature leaf drop, formation of multiple buds on defoliate branches, dieback of twigs, production of smaller and lopsided fruits. In greening infected plants incidence can be reduced by injecting tetracycline into trunk as well as spraying on the foliage.

Exocortis

It is destructive disease when susceptible rootstocks like Rangpur lime, P. trifoliate and citrange are used, to prevent exocortis use of virus free bud wood, use of disinfected budding knives and other tools.

Pests

Citrus trunk borer

Anoplophora versteegi is the most serious pest in north eastern India, Infestation can be reduced by prophylactic smearing of the trunk before onset of mansoon with Monocrotophos: Lime: Water (1:1:25). Infected tree whole should be spiked with wire and plugged with cotton socked in Nuvan or dimecron and then plastered with mud.

Aphids

Aphids cause severe curling of young leaves and stunting of twigs. Stimulate the growth of sooty mould fungus by secreting of honew dew. In India, four major spp. *Toxoptera aurantii, T. citricidus* (vector of Tristeza), *Myzus persicae, Aphis pomi.* Monocrotophos (0.025%), Phosphomidan (0.03%), Malathion (0.1%), Parathion (0.03%) spray can effectively control aphids.

Psylla

Diaphorina citri is widely distributed throughout the orient, it feeds all kinds of citrus, it sucks the sap from young leaves and tender shoots, it also a vector of citrus greening disease.

Psylla can be controlled by the spray of Monocrotophos (0.025%), Phosphomidan (0.03%).

Leaf miner

Phyllocnistis citrella damage both young and old plants. The infested leaves show chlorosis, leaf distortion, defoliation. It generally causes serpentine mines under the leaves. Spraying of Monocrotophos 0.03% can effectively control this pest.

Scales

Soft green scale (*Coccus viridus*), soft green scale (Coccus hesperidiums) spray of Parathion 0.03% can effectively control scales.

Lemon butterfly

Papilo demolens lay eggs on younger leaves and caterpillars feeds on, spraying of Malathion (0.01%).

Mealy bug

Psuedococcus species suck the plant sap, sever infestation causes defoliation and fruit drop, control- Tie a rubber band on the tree trunk in Oct-Nov. or frequent spray of Monocrotophos 0.02%

Mites

Red and green mite are important, cause greying distortion of leaves with mesophyll collapse, leading to partial or complete defoliation, pest control by malathion (0.05 % spray.)

Nematodes

Nematode are responsible for slow or spreading decline of citrus plantations, it may affect the growth of citrus trees by a) by injuring root bark, removing of plant nutrients during feeding, impairing normal growth and functioning of roots, possibly injecting toxic material in plant roots. Citrus nematode (*Tylenchulus semipenitrans*) is most destructive to citrus groves. Besides it some other nematode also found in citrus orchards like *Radopholus similis, Rotylenchulus reniformis, Pratylenchus coffeae, Meloidegyne javanica.* Soil application of Aldicarb 6kg/ha, 1000 ppm Carbofuran, Monocrotophos 1 g/ ha can effectively reduce nematode population.

Types of citrus decline according visual appearances or symptoms and their control

Quick decline
Slow decline
Spreading decline

Quick Decline

It occurs due to Tristeza virus plant may die within 1-3 weeks following symptoms we can identify infection of quick decline or virus.

Symptoms

Leaf- chlorotic leaf flecking, vein clearing, leaf cupping, corking of leaf veins, and stem pitting

Whole tree- The symptoms are similar to root injury. These symptoms include thinning of foliage, twig dieback, retardation of growth (stunted plants) and possibly tree collapse.

Trunk- Inside of the bark a honeycomb or stem pitting appearance can be detected with the unaided eye. In the trunks and limbs of larger trees, there sometimes is a bumpy or ropy appearance caused by the pitting.

Some of the infected trees decline overnight and dry up in 2-3 days.

Virus detecting techniques- DAS-ELISA, tissue-print ELISA, and PCR-based assays

Disease cycle (Quick decline/Citrus tristeza disease)

Virus the causal agent of disease named as Citrus tristeza virus (Closterovirus) caused Citrus tristeza disease. Two strains of tristeza, viz., the Virulent or fulminate and "avirulent" or mild have been recognized. Among the two strains of virus, one of which is an independent agent of stem-pitting, while the other causes seedling yellows.

Insect vector transmission of the virus by several insects viz. by *Aphis citricides, Toxoptera citricidus, Toxoptera aurantii,*

Aphis gossypii, Aphis craccivora, Aphis.spiraecola. Besides insects, the virus can be transmitted by budding, grafting and by means of dodder

Control of citrus tristeza virus or quick decline

Use of resistant rootstocks- Jattikhatti, Rangapur lime, Cleopatra mandarin, Troyer citrange or Swingle citromelo**.**

Cross protection technique

A mild strain inoculated in young seedlings grown in isolated nurseries, then these inoculated plants provide protection against virulent strain at main field.

CRISPR technology

"CRISPR" stands for "clusters of regularly interspaced short palindromic repeats." It is a specialized region of DNA with two distinct characteristics: the presence of nucleotide repeats and spacers. Repeated sequences of nucleotides — the building blocks of DNA — are distributed throughout a CRISPR region. Spacers are bits of DNA that are interspersed among these repeated sequences. A simple version of the CRISPR/Cas system, CRISPR/Cas9, has been modified to edit genomes. By delivering the Cas9 nuclease complexed with a synthetic guide RNA (gRNA) into a cell, the cell's genome can be cut at a desired location, allowing existing genes to be removed and/or new ones added.

RdRP (pathogen derived resistance)

Either intact or modified form of viral replicase or RNA dependent RNA Polymerase, this type of resistance can be divide in two broad categories like first functional or disfunction proteins are interferes with replicase enzyme complex and thereby disrupts the viral replication cycle. In other RNA based mechanisms that are related to the post transcriptional gene silencing.

RNA Interference is (RNAi) is a biological process in which RNA molecules inhibit gene expression or translation, by neutralizing targeted mRNA molecules. Historically, RNA interference was known by other names, including co-suppression, post-transcriptional gene silencing (PTGS), and quelling.

Coat proteins (p20 and p23, p20 and p25 intercellular level)- The *p25* coat protein (CP) gene of Citrus tristeza virus (CTV) was incorporated to Mexican lime plants and forty-two transgenic lines were produced, 25 containing the *p25 CP* gene of the severe CTV strain T-305 and 17 with that of the mild strain T-317. When plants propagated from each transgenic line were graft-inoculated with CTV T-305 oraphid inoculated with T-300, two types of response to viral challenge were observed: some lines developed CTV symptoms similar to those of non-transgenic controls, whereas others exhibited protection against the virus. Protection was efficient against non-homologous CTV strains and was generally accompanied by high accumulation of p25 CP in the protected lines, which suggest a CP-mediated protection mechanism in most cases.

Heat shock proteins

HSP-61, HSP-65- These proteins alter the binding process of virus with vector by disrupts in vectors forgut.

Biocontrol agents

In Biological control, predators, parasites, insect vectors are suppressed by biocontrol agents like *Aphelinus gossypii, Coelophera inaequalis, Pseudodorus clavatus. Coccinellid predator, Lady bird beetle, Cyptolaemus*

montouzieria are feeder of mealy bug. *Toxoptera aurantii and Aphis citricola suppress* by *Lysiphlebus testaccipes. Tamarixia radiata* anymphal parasite on citrus psylla. Citrus nematode suppresses by *Paecilomyces lilacinus.*

Slow decline

It is a gradual deteriorating process exhibiting loss of vigor, death of twigs and branches, reduction in yield and ultimate death in severe case, Due to poor nutrient, soil status and nematodes infection (*Tylenchulus semipenitrans*).

Above ground symptoms

Sparse foliage, exposure of bare crown limbs.

Dull gray-green leaves smaller than normal, Upright and cupped, drop early

Trees wilt often during mid-day, Reduction in number and size of fruits

Below ground symptoms

Small feeder roots do not develop properly causing slow decline.

Infested roots appear dark while healthy roots are creamish.

Control

Carbofuran @ 4 kg a.i./ha of soil just before flowering. Fenamiphos (Nemacur) 10.8-21.6 kg a.i./ha, aldicarb (Temik) 5.5-11 kg a.i./ha followed by light irrigation, Interculture of onion, garlic or marigold in citrus orchard. Incorporation of Neem cake @1kg per tree along with Carbofuran @ 2kg a.i./ ha in basin area. Use of resistant rootstocks- Swingle citrumelo (Citrus paradisi x P. trifoliata) is highly resistant.The egg-parasitic fungus, *Paecilomyces lilacinus.*

Spreading decline

Symptoms

Sparse foliage, Retarded terminal growth, weak root system, twig dieback, general thriftiness, leaves may wilt mid-day but show temporary rejuvenation after irrigation.

Trees may bloom profusely but bears only few small fruits, 90% of feeder roots are destroyed below 30 inches

Within the past 10 - 15 years of declining condition has appeared in various groves. This condition is called spreading decline.

It is mainly caused by *Radopholus similis.*

Control

*Paecilomyces lilacinus*is egg parasitoid can reduce nematode population (biological control agent), prevention and quarantine measures are the best strategy to overcome this type of decline**.**

A summary on management practices for citrus decline

Scientific cultivation- Adopting package of practices like maintaining proper spacing, regularly doing intercultural operations, spray of insecticides when needed, maintain balance of essential nutrients in soil and plants. Maintains soil fertility, using of disease free bud wood, clean equipments like knives, scissor etc.

Grow resistant species/ varieties- Troyer citrange, *Poncirus trifoliata* some other, mentioned above, selection of rootstock according desirable characteristics.

Use indexed planting material- For early detection of viruses like using of sweet orange

for detection of greening disease, it shows symptoms within 4-12 weeks after infection.

Use of appropriate rootstock- If we use compatible rootstock then citrus plantations will be long lasting, healthy with better yield.

Nutrient management- Maintains a balance amount of nutrients in the soil and plants, neither excess nor deficient I. 8:2:8:2:0.5:0.25:01(N: P_2O_5: K_2O: MgO: MnO: CuO: B_2O_3 respectively). In bearing stage khasi mandarin needed 300 g N: 250g P: 300g K.

Water management- Frequent irrigation should be needed in summer, in winter we can irrigate at 15-20 days interval. Grow only selected intercrops and management of weeds. Select right soil type and/ or manage it properly. Management of insect pest and nematodes, management of diseases (Fungal, Bacterial and Viral diseases).

Rejuvenation of declined citrus orchards through pruning, after pruning the Bordeaux mixture should be applied.

It is very dangerous complex problem we shouldn't neglect it, it affects all the citrus growing belts in the world. We should focus on scientific cultivation, aware farmers to citrus decline, how to prevent this this, how to control for that we should maintain soil fertility, nutrients balance (Integrated nutrient management), sanitation measures, we should use suitable rootstocks Integrated pest and disease management and focus on cross protection technique. Development of alteration genes against CTV binding to vector's forgut therefore vectors will be unable to carry viruses, CRISPR technology, RNA interference, RdRp. The introduction of site specific practices in citrus orchards, Precision citriculture requires to be popularized to identify, Sectorial occurrence of citrus decline, and accordingly develop the averting mechanism. Exploiting the utility of mycorrhiza and Rootstock breeding for resistance, cost effective technology for production of quality planting materials, Use of Genetic engineering technology.

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