Controlling Strategies of Citrus to Increase The Yield in The Country: A step Towards The Fight Against COVID-19

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Abstract

Citrus (oranges, lemon, mandarin, limes and tangarines) has significant nutritional value in human foods. It is rich source of vitamin C, sugar, organic acids, amino acids, minerals like Ca, Mg and various other phytochemicals (flavonoids, hesperidin etc) compounds that are responsible for good health. Citrus is growing in more than one forty countries of the world including Pakistan, Brazil, China, Mexico, USA, Spain and India. Viral infection and inflammation triggers the production of oxygen free radicals and these radicals severely damage the cells, however hesperidin and vitamin C is reported to counteract these damages. Intake of plentiful citrus fruits is one of amongst the many possible approaches to prevent from COVID-19 role of nutrition. Citrus fruit are very rich in important substances with a potential beneficial for health such as modulating the immunity and in protecting cells from oxidative stress related with infection. Flavonoids and hesperidin two major key compound found in citrus have affinity to treat Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Hesperidin has a low binding energy, both with the coronavirus “spike” protein, and with the fundamental protease that alters the primary proteins of the virus (pp1a and pp1ab) into the complex liable for viral replication hence these compounds could work as an antiviral function. Pakistan is amongst top ten citrus producers in the world. Citrus is one of the best commercial fruits of Pakistan cultivated on a large area, however, in Pakistan yield of citrus has been affected due to attack of insects and pathogens (nematode, fungal, bacterial and viral) which causes heavy losses both in quality and quantity. The purpose of this article is to focusing on the controlling the pathogens of citrus to boost citrus production in country as these beneficial plants are well known for its essential vitamin and flavonoid contents to control COVID-19.

Keywords: Citrus, Insects, Nematodes, Fungus, Yield, COVID-19

1. Introduction

The agriculture sector plays vital role in Pakistan’s economy. It contributes 18.9 % to Gross Domestic Production of Pakistan. Agriculture provides employment for 42.3% of the country’s people, and 75% of rural population is dependent on agriculture in Pakistan (Pakistan economic survey 2017-2018). citrus production is very important in Pakistan’s agriculture industry, citrus is one of the major fruit of the world due to its popularity and nutritional value in human foods. The generic name originated from Latin, where it specifically referred to the plant now known as Citron (C. medica), derived from the ancient Greek word for cedar (kedros) [1]. The commercially available citrus species are oranges, lemon, mandarin, limes and tangarines. Citrus fruits are grown in tropical and sub-tropical regions (35°N and 35°S) latitude in north [2,3]. Citrus is growing in more than one forty countries of the world. Main citrus growing countries of the world are Brazil, China, Mexico, USA, Spain and India. Pakistan stands among top ten citrus producing countries of the world (Source: FAOSTAT). Kinnow (Citrus reticulate) is the major species in Pakistan among other citrus species with 95% share. Punjab shares 94% and 96% in area and production of citrus, respectively [4,5].

Citrus is extreme necessity for human health because of its high nutritional value and a rich source of vitamin C, sugar, organic acids, amino acids and minerals like Ca and Mg. In Pakistan average yield is 2.36 million tonnes, grown over 199,000 hectares and having export of 282,000 tons per year and fetching 7,313 million rupees [6,7]. On the other hand the
potential yield of citrus is 12-15 tons per hectare and hence there is a big difference between its average and potential yield in Pakistan. It is because of the reason that citrus has been attacked by many insects and pathogens (nematode, fungal, bacterial and viral) causing heavy losses both in quality and quantity [8,9].

2. Effect of Nematodes on the Yield of Citrus Fruit

Citrus fruit nematodes are worm like, tapering on both ends and have all main well developed physiological systems except circulatory and respiratory system. Many different genera and species of nematodes are involved to infest citrus plant [10]. These include Root-knot nematodes (*Meloidogyne* spp); Sting nematodes (*Belonolaimus* spp); Stubby-root nematodes (*Trichodorus* spp); Root-lesion nematodes (*Pratylenchus* spp); Cyst nematodes (*Heteroderma* spp); Awl nematodes (*Dolichodorus* spp); Stunt nematodes (*Tylenchorhynchus* spp); Lance nematodes (*Hoplolaimus* spp); Spiral nematodes (*Helicotylenchus* spp; *Scutellonema* spp); Ring nematodes (*Crinemoides* spp); Dagger nematodes (*Xiphinema* spp); Bud and leaf nematodes (*Aphelenchoide* spp); Reniform nematodes (*Rotylenchulus* spp); *Tylenchulus semipenetrans*; *Paratylenchus* sp.; *Psilenchus* sp.; *Radopholus* sp.; *Xiphinema* sp; *Zygolotylenchulus*; *Longidorus*; *Alamidsp; Araeolamidsp; Cephalobidsp; Diplogastridsp; Monhysteridsp; Mononchidsp; Plectus; *Rhabditids*. Citrus nematode inflicts damage to over 50 species of citrus causing yield loss approximately 43.3% worldwide [11]. Plant parasitic nematodes have stylet which penetrates into host plant and punctures its tissues for the absorption of nutrients. These live in the soil and attack the roots of plants causing production problems occur as a result of root dysfunction, reducing root volume and forcing reduced efficiency of water and nutrients uptake. Plant parasitic nematodes possess non-segmented microscopic body with cylindrical shape. The host range of these nematodes, as with others, includes most if not all of the commercially grown vegetables within the state. Yield reductions can be extensive but vary significantly between plant and nematode species.

The first report of an association between a nematode and citrus was reported by [12]. *Tylenchulus semipenetrans* was discovered by Hodeges in 1912 on roots of citrus trees in southern California. The citrus root nematode (later referred to as citrus nematode) was first reported by Thomas in 1913. Cobb described *T. semipenetrans* as the causal agent of citrus slow decline in 1913. In Pakistan *T. semipenetrans* firstly reported in [13]. [14], reported citrus root nematode as major contributor of decline of citrus plantation after surveying the major citrus growing areas in Punjab. Several nematodes are beneficial for citrus i.e. associated with [15]. Few of these are of economic importance (*Tylenchulus semipenetrans*) causing 'slow decline' of citrus, whereas most nematode species may cause serious damages to mature citrus. Incidence of *Tylenchulus semipenetrans* can be reduced by spreading out/growing citrus into new geographical areas. Nematodes parasitism cause gradual reduction in tree quality so that infested trees are smaller over a long period, become less vigorous and may yield apparently less fruit [16]. Symptoms of nematodes infestation include poor root development, smaller chlorotic leaves, and slower development. However, in saline environment, undue sodium may accumulate in leaves [17] and wilting can occur earlier during periods of water stress. Loss of integrity at the epidermis and at feeding sites in the cortex causes feeder roots putrefy quicker resultanty attacked by secondary organisms [18] causing lesions on lightly infected roots, while heavy infections result in cortical sloughing and root death. The life cycle of *T. semipenetrans* regulated by host phenology, interrelated with geographic variation and temporal changes in the soil environment. Many studies reported one [19], or three [20] distinctive periods of active population growth per annum. The economic life of citrus trees in some countries is about fifty years and in some cases in good positions they can live 100 years. Due to its geographical location, climatic conditions and soil type, citrus population of Pakistan is more prone towards nematode infestation. In Pakistan, the normal age of citrus tree is considered to be 25 years. The reason is that citrus orchard, in majority of cases starts declining. *T. semipenetrans* infestation of citrus was first reported in Faisalabad (Punjab) in 1962 (Brown, 1962). According to different studies, estimated yield losses due to *T. semipenetrans* to be in the range of 10% to 30% depending on the level of infestation [21]. However, the nematode can be of great economic importance in orchards where the fruit has marketed fresh rather than processed as *T. semipenetrans* often reduces fruit size [22].

3. Symptoms of *T. semipenetrans* infestation

The disease caused by *T. semipenetrans* referred to a “slow decline” which showed the symptoms on citrus trees. There is continuous decline in growth of tree and reduction in fruit size. As the population of nematode increases in the soil and e roots, over all yields becomes low every year. When young trees were planted in land harshly infested with nematode, damage symptoms appeared more rapidly [23]. Leaves Of seriously infected trees showed deficiency of nutrients concentrations such as potassium, which showed the importance of fertilization [24]. During the time of water stress, fibrous roots were less abundant so that wilting occurred and dropping of leaves caused thinner tree canopies in high infested trees [25]. Feeder roots rotten faster due to damage of integrity at the epidermis and at feeding sites in the cortex, resulting in attack by secondary organisms [26]. This may be expressed as lesions on lightly infected roots, while heavy infections cause cortical sloughing and feeder roots became slightly thicker than healthy roots and showed a dirty appearance, due to soil
particles that adhere to gelatinous egg masses on the root surface. Symptoms depend on the favorable environmental conditions and cultural practices for citrus health. Infested trees under optimum conditions may seem healthy [27,28].

4. Effects of Other Pathogens on Citrus Fruit

These requirements involve pests of quarantine concern for the importing countries, which risks are diminished by the application of phytosanitary measures. Gamma radiations are used as sanitation treatment with no significant effect on fresh fruit quality. The Gamma radiations as quarantine treatment is a phytosanitary measure under the IPPC (International Plant Protection Convention), approved by an international standard. Viral Species Different viral species caused various illnesses in citrus plant. Citrus mosaic disease is caused by Satsuma dwarf-related virus [29,30] Bud union crease caused by Virus for some combinations; Citrus leaf rugose caused by Citrus leaf rugose virus (CLRV) [31]. Citrus yellow mosaic disease caused by genus Badnavirus [32,33]. Crinkly leaf and Infectious variegation is caused by Citrus variegation virus [34]. Navel infectious mottling caused by Satsuma dwarf-related virus whereas CPsV (Citrus psorosis virus) cause Psorosis and SDV (Satsuma dwarf virus) cause Satsuma dwarf [35-38]. Genus Capillo also known as Citrus tatter leaf virus cause Tatter leaf (citrange stunt) and CTV (Citrus tristeza virus) of genus Clostero virus cause Tristeza [39-41]. Control strategies for these viral diseases include evading or keeping out of these pathogens from the area. Use of certified bud wood, and/or eradication or suppression of these pathogens, use of tolerant or resistant cultivars and/or cross protection [42]. Cultural practices that reduce infection are also helpful to control viral diseases [43]. However success of control strategies depends on knowledge of the causal agent of the disease, symptoms and mode of propagation etc

5. Bacterial Species

Xanthomonas euvesicatoria pv, citrnelo, species of proteobacteria cause bacterial spot; Pseudomonas syringae cause Black pit (fruit) and Blast; Xanthomonas citri pv, citri cause Citrus canker ; Citrus variegated chlorosis is caused by Xylella fastidiosa and Huanglongbing is caused by Candidatus Liberibacter asiaticus and Candidatus L. africanus Bacterial spot of citrus plant caused by bacterium Xanthomonas alfalfa. Bacterium spreads tree to tree via wind-blown rain, dropping dew or overhead irrigation through natural pores on leaves and/or the lenticels on bark [44]. It affects mainly trifoliate orange and its hybrids. Pathological lesions formed are flat or sunken and not raised. Prevalence of pathogen is less when the weather is hot and dry. To control bacterial diseases preventive measures along with biological treatments are usually advised. Copper based sprays and/or application of spray together with oxytetracycline/streptomycin and/or the chemical mancozeb may be used to control the disease. However chemical sprays and medicines should always use within recommended prescribed dose to avoid damage to leaves and the development of resistance.

6. Fusarium Spp.

Citrus fruit begins to expose a critical period after harvest because numerous pathogens such as fungi (i.e. Penicillium, Elsinoe spp.) may cause fruit rots during this period [45]. Thirteen different types of fungi including Fusarium spp., Penicillium sp., Curvularia sp., Aspergillus spp., Alternaria sp., Guignardia sp. Diplodia sp. and Colletotrichum sp. Fusarium spp are almost found in every region and is mostly soil born pathogen. It has been estimated to affect 11.6% of mandarin trees and caused 39.6% loss in fruit yield [46]. F. solani disease of citrus was reported to attack all citrus varieties [47,48].

Fusarium solani causes two types of diseases, dry root rot and chronic feeder root rot and both cause citrus slow decline [49]. Dry root rot of citrus caused by F. solani(Mart.) is one of the most serious diseases of citrus attack orchards especially that cultivated in new reclaimed lands. F. solani produces two types of root rots on citrus trees. Firstly, dry root rot limited to the crown and roots scaffold. Secondly fibrous and feeder root rots associated with continuing decline of the canopy, leaf curl like wilting, dieback, defoliation; Fibrous roots become soft, and seem like water soaked, slough their cortex easily by hand[50-53].

F. solani caused fibrous and scaffold root rot on citrus at various extents in the field [54-56]. F. solani entered the root system and the entire root turned reddish-purple to greyish-black [57]. The wood below the bark has no rottening but may be marked dark [58], and when the cross section of an infected trunk was examined a greyish-brown discoloration in the wood tissue could be detected [59]. Studies by [60,61], showed that the fungus caused on inoculated seedlings symptoms that are identical to those on field trees. On adult plants, the most conspicuous symptom above the soil level is a fatal collapse of the tree [62]; leaves suddenly wilt and dry up in a few days, remaining attached on the tree. Normally, the course of the disease is chronic and symptoms look like those provoked by other root rot agents. Trees showed a lingering decline, progressing for years, with the only initial symptoms being a slight wilt under dry conditions and poor vegetation flush [63]. At more advanced stage of the decline, the canopy has a thin and sparse appearance.
7. Interaction of Fusarium with Nematode

Fusarium has most important group of fungus because Fusarium caused many serious diseases of plants. Citrus roots which are infested with the nematode are also mostly infested with [64]. T. semipenetrans interacts with soil organisms which can regulate or influence fusarium spp. population. In fact, Walker and Morey [65] showed that the nematode may increase the pathogenicity of F. solani under favourable conditions. Phytophthora nicotianae is a virulent pathogen of citrus roots. It was shown that pre-infection of citrus roots by T. semipenetrans can reduce the rate of infection by [66]. There are some evidences that growth of the fungus is inhibited in the vicinity of the nematode eggs, but a mechanism has unknown. Dry root rot caused by Fusarium spp. has a serious threat in some countries [67]; when it remains an interaction with root rot disease caused by Phytophthora spp. [6], and with citrus nematode T. semipenetrans [36]. Dry root rot of citrus occurs on trees that usually are weakened by some other factor (root damage caused by Phytophthora, over-watering, poor soil drainage, excess fertilizer, heat stress and root injury due to plowing, herbicides or nematodes [68].

During COVID-19 pandemic, the significance of citrus fruits has been increased exponentially due to its increased demand. Use of citrus fruits (oranges, lemon, grapefruit etc.) is generally recommended by health officials to improve the immunity as one of the preventive measures of COVID-19 [64, 65]. Citrus fruits such as orange are enriched with 9.9 g of carbohydrates (45 kcal of energy per 100 g), 0.7 g of proteins, 0.2 g of fats, [69,70]. Two major components found in citrus fruits are hesperidin and vitamin C which have multiple biological actions which appear to be effective to respond the cells to modulate the systemic immunopathological phases of COVID-19 Infection [71-74].

Citrus fruits are the rich source of flavonoids (phenolic compounds) which are associated with immunomodulatory as well as anti-viral activities. Naringenin is a flavonoid found in citrus fruits [75,76]. Its anti-viral activity might be helpful to cope up with viral infections including COVID-19 by either regulating the production of cytokines or preventing the replication of viruses [77,78]. Among Citrus fruits, grapefruits, pummelo and oranges have excess amount of naringenin [64,74]. It was reported that naringenin may prevent COVID-19 either by binding with angiotensin converting enzyme-2 (ACE-2) receptor that is the possible binding site for novel corona virus or by attenuating the inflammatory responses [67; 66; 75]. Another study also reported that lemon oil contains limonene as an active ingredient and it was found to down regulate the ACE-2 receptors [79]. However, further preclinical, clinical and epidemiological studies are required to verify the hypothesis.

The best approaches to prevent COVID-19 is to boost the immune system of the body through optimal nutrition intake [70,71]. Citrus fruits are known to up regulate the immune response by regulating the production of cytokines (signaling molecules) and hence, helpful in the prevention and control of viral infections. These are considered the economical nutritional therapy to fight with COVID-19 global threat [80,81].

Conclusion and Recommendation

Citrus is one of the important food crop of Pakistan that play pivotal role in the economy of Pakistan. However, in Pakistan yield of citrus is affected due to attack of insects and pathogens (nematode, fungal, bacterial and viral) which causes heavy losses both in quality and quantity. It is strongly recommended that effect/interaction of these insects and pathogens on performance of citrus fruit must be evaluated. For this purpose research should be conducted to investigate prevalence and pathogenicity of these pathogens. These pathogens should be identified at species level by molecular markers and control strategies should be devised to prevent the fruits from disease.

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