



# Management of Fusarium Wilt of Tomato (*Fusarium oxysporum* f. sp. *lycopersici*) and Related Soil-borne Diseases using Eco-friendly Methods: A Review

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

Tomato is rich in vitamins, minerals and contains anti-oxidant compounds, like vitamin C and lycopene that has significant role in human health as it has anti-cancer properties. Tomato production is significantly affected by several factors amongst which Fusarium Wilt is one of the major and widespread fungal soil borne diseases in Nigeria. Difficulty in controlling this disease is attributable to long survivability of the pathogen and its existence in diverse pathogenic races. Conventional strategies, such as the use of resistant cultivars and synthetic fungicides, are not completely effective in managing tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* and because of unintended effects of chemicals on the environments its application is highly cautioned. This paper has examined many non-pesticide approaches such as application of bio-control agents,

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use of suppressive soils, composts/vermicomposts and their extracts, animal manure and urine, soil solarization, botanical extracts, good agricultural practices and integrated disease management strategy to control Fusarium wilt and other related soil borne diseases. It is worth noting that no single method can effectively control such diseases. In order to achieve a sustainable and eco-friendly disease management strategy the use of integrated approach is highly recommended. This review will provide farmers with diverse methods to be integrated as management package for Fusarium wilt and related diseases.

**Keywords:** *Bio-solarization; bio-fumigation; compost; eco-friendly disease management; fungicides; Fusarium wilts; soil-borne diseases; suppressive soil; tomato.*

## 1. INTRODUCTION

“Tomato (*Solanum lycopersicum* L.) is a vital component of daily food and is consumed as unprocessed fresh fruits as well as in various types of processed products” [1]. “It is cultivated in all countries either in fields or in protected culture” [2]. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen (FOL) is one of the most significant diseases affecting tomato worldwide [3,4,5,6], especially in the acidic sandy soils of tropical regions [7]. “The disease has become a limiting factor to the sustainable development of tomato production due to the long-term survival of the chlamydospore in the plant debris of the soil” [8].

“*Fusarium oxysporum* f. sp. *lycopersici* as a soil-borne pathogen can survive indefinitely in the absence of host in infested soil as chlamydospores, and its germination is triggered by the presence of tomato root” [9,7]. “Sequential steps involved in the infection process of FOL is summarized as root recognition through host-pathogen signals, attachment to surface of root hairs and hyphal propagation, invasion of the root cortex, and vascular tissue and differentiation within xylem vessels and finally oozing of toxins and virulence factors” [7]. “This is followed by colonization of the vascular tissues leading to disease development that led to wilting of the host plant” [10,11].

“Fusarium wilt causes great losses on the susceptible varieties of tomato especially when soil and air temperature are high during the warm season” [12]. The pathogen inflicts a substantial yield loss ranging from 25 to 55% [13] and up to 90% is reported under optimal infection conditions [14]. In Nigeria, the disease causes serious yield losses up to of 50% [15], especially in the Northern parts of the country. “The pathogen is controlled by disinfecting the soil with methyl bromide, chloropicrin or metham sodium and systemic fungicides such as

benomyl, thiabendazole and thiophanate. However, sustainable use of fungicides in FOL management is difficult due to development of resistant isolates and damaging effects on the natural environment, the agro ecosystem and human beings” [16]. This stimulated the development of alternative methods as disease management strategies. These methods are briefly summarized as Follows:

## 2. USE OF BIO-CONTROL AGENTS

Biocontrol agents had been reported to be an effective method used in controlling plant diseases [17]. They have been reported to be used in the control of Fusarium wilts for many crops including tomato, cucumber, melon, strawberry, banana and carnation [18]. Bio-control agents such as non-pathogenic *F. oxysporum* [19,20]; hypovirulent binucleate *Rhizoctonia* [21], *Gliocladium virens* *Trichoderma hamatum*, *Burkholderia cepaci*, *Pseudomonas fluorescens* [22], *Bacillus subtilis*, *Streptomyces pulcher* [23], *Bacillus polymyxa* [24] and *Enterobacter cloacae* [25] have been reported to control Fusarium wilt disease. Use of Mycoparasites such as *Trichoderma harzianum*, *T. viride* and *T. hamatum* as bio-control agents in tomato field significantly controlled tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* [26].

“Suppression of Fusarium wilt using biocontrol agents has been achieved through interactions among the plant, the pathogen, the biocontrol agents, the microbial community around the plant and the physical environment” [27]. Hydrolytic enzymes of antagonistic microorganisms have been considered to play an important role in the biological control of plant pathogens. Many enzymes have been isolated from various strains of *Trichoderma* Species [28], *Gliocladium vixens* [29], *Paenibacillus* and *Streptomyces* species [30], and their activities were assayed and found effective in controlling Fusarium wilts. This is

accomplished through competition; secretions of antibiotics, parasitism and induced resistance [31].

### 3. USE OF SUPPRESSIVE SOILS

“Soil disease suppression is the reduction in the incidence of soil-borne diseases even in the presence of a host plant and inoculum in the soil, mainly attributed to diverse microbial communities present in the soil that could act against soil-borne pathogens in multifaceted ways” [32]. Many groups of microorganisms found in the soil are potential biological control agents which suppress Fusarium wilt of tomato [33]. Soil has untapped and several potential biocontrol agents that have shown high antagonistic activity against several soil-borne pathogens [34]. The soil-inhabiting antagonists were found to be effective suppressant to Fusarium wilt of tomato and these include; non-pathogenic *F. oxysporum* [35], *Trichoderma*, *Rhizoctonia*, and *Gliocladium* [36], *Bacillus* species and *Streptomyces* species [37], *Pseudomonas* spp. [38]. Biological mechanisms through which the disease-suppressive soils control the disease development are illustrated in Fig. 1.

### 4. USE OF COMPOSTS/VERMICOMPOSTS AS ORGANIC AMENDMENTS

Organic amendments showed antibacterial and antifungal activity against soil-borne and foliar pathogens. Bananomi et al. [39] reported that extensive application of composts controlled several soil-borne pathogens (*Rhizoctonia solani*, *Sclerotinia* spp., *Pythium* spp., *Verticillium dahliae*, *Fusarium* spp., *Phytophthora* spp., and *Thielaviopsis* sp.) which were responsible for causing wilting, damping-off and decaying in many horticultural crops. Liu et al. [40] reported the use of organic amendments as an eco-friendly practice that promote soil fertility and suppressiveness against a wide range of soil-borne pathogens. Many types of composts were reported to have suppressed Fusarium wilts in different crops [41,42]. The efficacy of composts in managing soil-borne disease varied according to Termorshuizen et al. [43], but it is still considered a suitable method of controlling soil-borne diseases [44]. Avilés et al. [45] attributed “the ability of compost to suppress plant pathogens to various roles played by microbes in composts which might be directly responsible for antagonistic interactions with pathogens and/or for the induction of systemic resistance in host

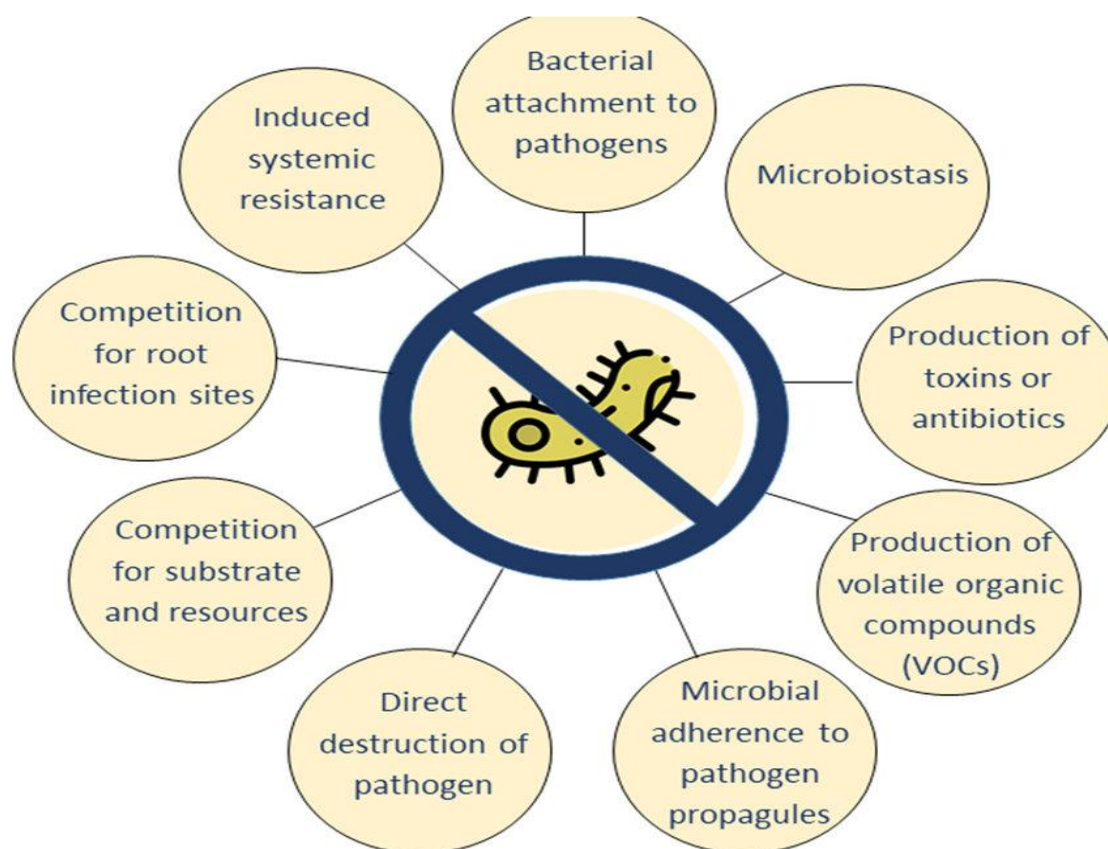
plants” [46]. “Microbial activity during compost maturation indirectly contributed to the formation of natural bio-stimulants and humic molecules which triggered bio-control agents to suppress soil pathogens” [47].

Despite the potential of composts to suppress soil-borne pathogens in general, the success of the application of certain composts against a particular pathogen is not predictable [48], this is one of the major limitations of compost application in soil borne disease management. In an effort to increase harnessing of compost microbiomes for plant protection, Lutz et al. [48] proposed an integrated systems approach (Fig. 2). They further reported that outcomes of the systems approach may include identification and isolation of new biocontrol strains and their mode of action, development of microbial consortia with suppressive superior to single strains, development of diagnostic tools to allow a targeted application of composts against soil-borne diseases, and the development of strategies to selectively promote key microbial organisms in composts. Compost application to agricultural fields is an excellent natural approach, which can be taken to fight against plant pathogens and its application is also an environmentally friendly alternative to chemical use [49] and improve the soil health and its nutrient levels.

“Use of organic matter in the form of vermicomposts has been recognized as having considerable potential as soil amendments for improving yield and managing tomato wilt caused by *FOL*” [50,51]. “Vermicomposts which are products of organic matter degradation through interactions between earthworms and microorganisms, accelerates the rates of decomposition of the organic matter, alters the physical and chemical properties of the material, and lowers the C: N ratio, leading to a rapid humification” [52]. This increases the surface area of the materials for colonization by beneficial microorganisms [53].

### 5. USE OF COMPOST/VERMICOMPOST EXTRACTS

“Compost extracts are liquid extracts derived from mature composts, suitable for application to soil and/or as Foliar spray” [54]. “It contains organic and inorganic soluble nutrients, and a large number of organisms including fungi, bacteria, protozoa and nematodes” [55]. Compost extracts are also known as compost



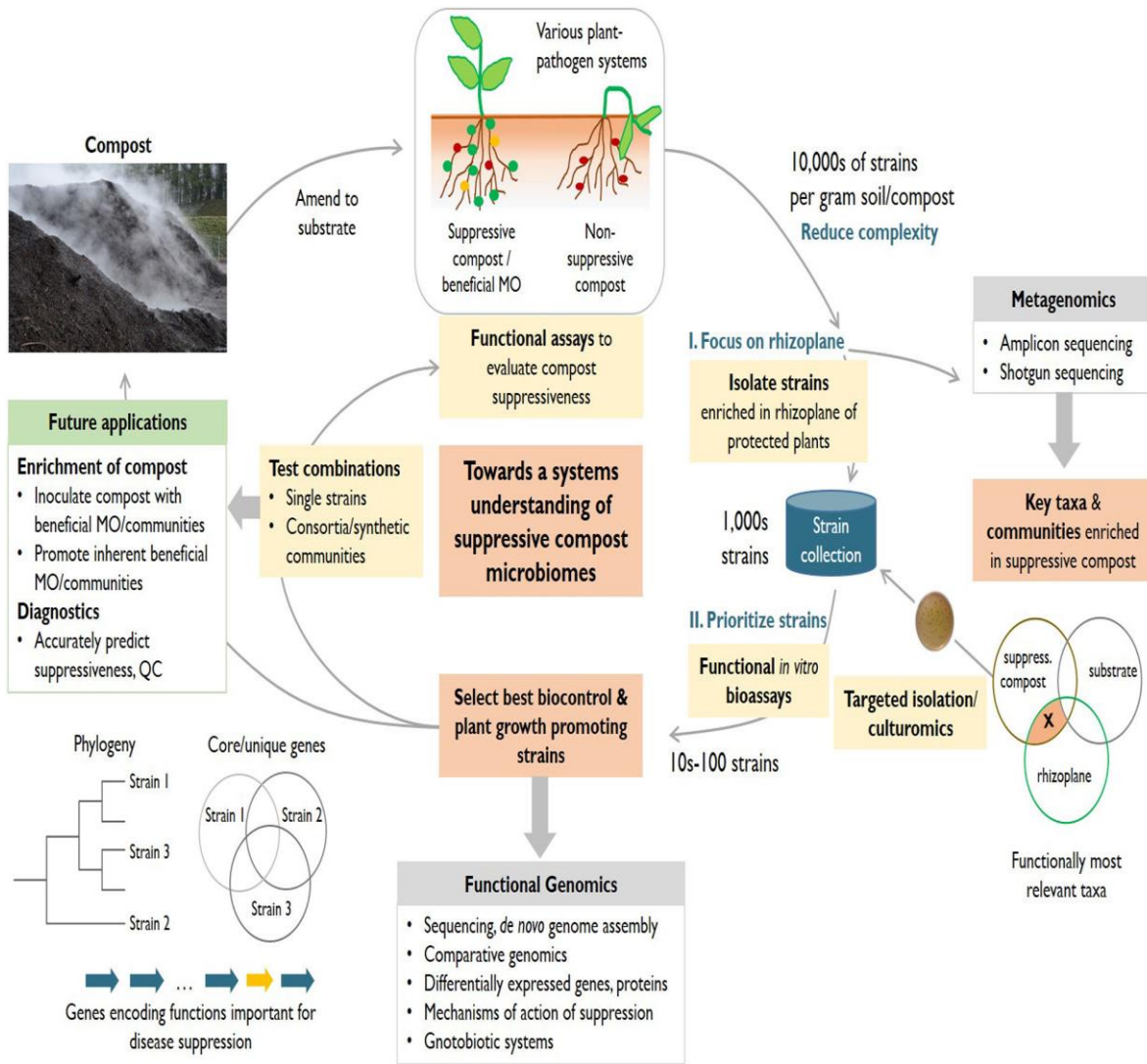
**Fig. 1. Possible mechanisms of disease-suppressive soil a close view**

Source [Jayaraman et al., 2021]

teas [56]. Divers [57] reported that “the active compounds identified in compost extracts include bacteria (*Bacillus*), yeasts (*Sporobolomycetes* and *Cryptococcus*) and fungi, as well as chemical antagonists such as phenols and amino acids”. “Water extracts prepared from composted organic matter had been used by farmers for many years for their perceived beneficial effects on plant health” [58,40]. Yohalem and others [59] reported that “compost extract reduced the severity of Foliar diseases such as powdery and downy mildews of grapes caused by *Plasmopara viticola* and *Uncinula necator* (syn. *Erysiphe necator*) respectively, gray mould of straw berries (*Botrytis cinerea*), and late blight of potato (*Phytophthora infestans*)”.

“Microbial populations in compost extracts are considered the most significant factor contributing to disease suppression” [60]. “However, there is a limited understanding of the microbial species composition, how these organisms survive on plants and the role of microbial diversity in the efficacy of the extracts. Results obtained from several studies suggested that antibiosis, which is a mechanism of

suppression might be responsible, due to retention of suppressive qualities from the filter or heat sterilized extracts” [59]. “There is evidence that antibiotic metabolites present in compost extracts originate from the compost source” [61]. Al-Dahmani and his co-workers [62] reported “significant but inconsistent control of tomato bacterial spot (*Xanthomonas vesicatoria*) with cow manure; pine bark; and yard waste compost extracts”. Hointink and co-workers [63] reported that the mechanisms of action exhibited by compost extracts in suppressing plant diseases appear to be antibiotic, competitive, parasitic and through induction of resistance. However, the efficacy and the suppressive ability of compost extracts depend on: age of compost, source of compost, type of target pathogen, method of preparation, meteorological condition, timing and frequency of application [64]. Research studies showed that aqueous extracts of vermicompost and/or organic compost were effective in inhibiting mycelial growth of *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium oxysporum* f.sp. *lycopersici* [65,66].



**Fig. 2. An integrated systems approach to enhance understanding of the microorganisms responsible for the disease-suppressive effects of compost in various plant pathogen systems.**

Source: [48]

## 6. USE OF ANIMAL MANURE AND URINE

Basak et al. [67] studied the efficacy and *in vitro* activities of cow urine and dung for controlling wilt caused by *F. oxysporum* f.sp. *cucumerinum* of cucumber and *F. solani* f.sp. *cucurbitae*. Cow dung solution (20 grams, peeled and sliced potato- 200 grams, dextrose 20 grams, agar- 20 grams and distilled water-1000 mL) and urine (500 mL and distilled water 500 mL) showed 80 - 84% and 100% inhibition of wilt pathogens, respectively.

Ashlesha et al. [68] reported “effective control of damping-off of okra and root rot of pea incited by *R. solani* using fresh cow dung, cow urine and

cow milk based preparations”. Sinha et al. [69] studied “the antifungal properties of vermicompost and vermivash against soil borne pathogens (*Pythium ultimum*, *R. solani* and *Fusarium* sp.) and recorded 51-72% inhibition in mycelial growth of the pathogens”. Similarly, Sang et al. [70] reported “the reduction in mycelial growth of *Phytophthora capsici* and *Colletotrichum coccodes* in pepper and *C. orbiculare* in cucumber by water extracts of compost”. Sugha [71] evaluated “the antifungal potential of panchgavya (combination of five dairy products) against *R. solani*, *S. rolfsii*, *F. solani*, *S. sclerotiorum* and *Phytophthora colocasiae* and advocated that the mycelial bits dipped for 6 h in panchgavya caused complete

suppression of mycelial growth of *R. solani* and other pathogens". Dogra [72] also reported "the antifungal activity of panchgavya against major soil-borne pathogens comprising *F. oxysporum* f.sp. *pisii*, *R. solani*, *S. solfsii* and *S. sclerotiorum*".

## 7. BIO-FUMIGATION

"The term bio-fumigation refers to the allelopathic effects of biocidal glucosinolate (GSL) hydrolysis products, principally isothiocyanates (ITCs) on soil organisms" [73]. The term "bio-fumigation" was coined to describe the suppression of soil-borne pathogens by compounds released from *Brassica* tissues, and implies a greater reduction in the load of pathogen inoculum than that resulted from the simple absence of a host. Bio-fumigation denotes the application of plant materials to treat fields infected with diseases or nematodes [74]. Trindade and Aries [75] defined bio-fumigation as the suppression of soil-borne pests by biocidal compounds of plant origin such as isothiocyanates and polyphenols. It is therefore, an intentional use of bioactive plants and other organic materials that aid in reducing populations of plant pests in soil, mostly *Brassicaceae* and *Allium* species [76].

"Suppression of soil-borne pests by bio-fumigation was achieved due to the presence of biocidal compounds; particularly isothiocyanates released from brassicaceous rotation and green manure crops when the glucosinolates (GSLs) in their tissues were hydrolysed in soil" [77]. "Some hydrolysis products, particularly the ITCs were known to have broad biocidal activity including insecticidal, nematocidal, fungicidal, antibiotic and phytotoxic effects" [78]. Biofumigation also denotes the application of agronomic practices which involves the use of volatile chemicals (allelochemicals) released from decomposing plant tissues or using a specific plant species containing identified toxic molecules to suppress soil-borne pests [79]. Apart from brassicas, plants from *Caricaceae*, *Moringaceae*, *Salvadoraceae* and *Tropaeolaceae* families were also known to have bio-fumigant properties [80,81]. Arnault et al. [82] reported the ability of *Brassicaceae* and *Alliaceae* family in producing many sulphur-containing volatile compounds arising via cleavage of certain S-alk(en)yl cysteine sulphoxides which could act on a variety of soil borne pests, including fungi, bacteria and nematodes.

Bio-fumigation greatly reduced pesticide application making farming cheaper and safer as

it added organic matter to the soil, leading to increased soil aeration, water infiltration rates and soil water holding capacity [83]. Bio-fumigation was also reported to have increased soil porosity if used as a green manure and added more organic carbon to the soil thereby increasing the activity of soil fauna and flora [84]. Most studies on bio-fumigation have been done using brassicas [85], plant families like *Alliaceae*, *Moringaceae* and *Caricaceae* also possess bio-fumigant properties and could therefore be used in bio-fumigation [83]. The liberation of volatile compounds from decomposing crop residues generally occurs within a few days after their incorporation in moist soil following decomposition of bio-fumigant plant tissues which principally release isothiocyanates, in addition to thiocyanates, nitriles and oxazolidinethiones [86]. Isothiocyanates are released following tissue damage when endogenous myrosinase enzymes hydrolyze glucosinolates which are produced by plants as secondary metabolites [12]. Degradation of tissues of *Alliaceae* releases sulphurous volatiles such as thiosulfines which is converted into disulphides that have biocidal properties against fungi, nematodes, bacteria and other pathogens [87].

Bio-fumigant crops can be grown as cover crops or intercrops which could be slashed and ploughed under at flowering [88]. Besides growing the brassicas as green manure crops, they can be formulated into either a brassica cake or powder which can be incorporated into the soil or may be used as mulch [89]. It was also reported that bio fumigation enhanced soil saprophytic activity by microbes like *Streptomyces* sp. which act as agents for the induction of plant resistance to diseases like those caused by *Rhizoctonia* sp. [90]. Biofumigation with poultry manure and cabbage residues has been reported to control *Fusarium* wilt of tomato and therefore recommended to be applied as bio-pesticides and also as bio-fertilizer for improving yield on *Fusarium* wilt infected tomatoes [91].

## 8. SOIL SOLARIZATION

Soil solarization is hydrothermal soil disinfestations process that utilizes clear plastic mulch to trap solar radiation in moist soil during the hottest periods resulting in increased soil temperature to levels that can be lethal to soil-borne pathogens [92,93,94,95,96,97]. Pokharel and Hammon [98] and Siddiqi et al. [98] reported

that high temperature generated during solarization induced changes in soil volatile compounds that were toxic to soil pests and pathogenic organisms. This method has attracted the interest of scientists in many warm-climate countries because of its effectiveness, simplicity, and safety for humans, plants, and the environment [98,99]. Soil solarization as a natural hydrothermal process of disinfecting soil is accomplished through passive solar heating. Gelsomino et al. [100] also reported that reduction of soil-borne inoculum of plant pathogens achieved by direct thermal inactivation at soil temperatures ranging from 40°C to more than 60°C as the primary function of solarization to manage fungi, bacteria and nematodes.

Researchers have reported the use of soil solarization as eco-friendly method against fungal pathogens such as *Verticillium* spp., *Fusarium* spp. and *Phytophthora cinnamomi* and bacterial pathogens such as *Streptomyces scabies*, *Agrobacterium tumefaciens*, and *Clavibacter michiganensis* [101,102]. Chen et al. [103] revealed the ability of soil solarization to increase the release of soluble nutrients such as inorganic N forms, extractable P and K, available cations, and dissolved organic matter resulting in improved vegetative plant growth and yield. Soil solarization is an attractive alternative method to replace the use of methyl bromide that was phased out in 2015. Fuentes et al. [104] opined that soil solarization alone might not consistently be effective to control soil-borne pathogens and pests, but its efficacy could be increased if combined with cultural, biological and/or chemical methods. The method could be used singly or in combination with other methods to effectively manage soil-borne diseases [105].

## 9. BIO-SOLARISATION

Combining solarization with the incorporation of plant residues in the soil produces a more consistently suppressive and sometimes eradicated effect on soil-borne fungi, bacteria, nematodes, weeds and pests rather than either treatment used alone [97]. Soil solarization as reported by Eshel et al. [106] could be improved by combining it with other control measures. Managing *Fusarium oxysporum* f. sp. *lycopersici*, the causal agent of tomato wilt with solarization alone was not effective because the soil-borne pathogen was heat tolerant [108]. Several researchers have reported the potential of improving pathogen control and expansion of

spectrum activity of biocontrol agents by integrating soil solarization with organic amendments [94,107]. Integrating soil solarization with plant residues proved to be good alternative in managing soil-borne pathogens especially under a situation where the use of organic amendment alone proved to be ineffective [109].

Many studies have demonstrated the effectiveness of combining solarization with biofumigation by covering and heating soil containing Brassica residues which produced deleterious effects on soil-borne fungi and nematodes far better than where the residues were simply incorporated into natural field soil [110]. Combining soil solarization and biofumigation has been a good measure in combating diseases caused by soil-borne pathogens such as Fusarium wilt of tomato [111]. Bio-solarization is also known as anaerobic soil disinfestation (ASD), described as biological process of disinfecting the soil by creating its condition anaerobic using simply decomposable amendments such as rice bran, fresh crop residues and soybean flour by covering it with polypropylene and water it to saturation [112]. The soil amendments used in ASD provide the substrate for speedy microbial growth [113]. Application of water on the organic amendment, covering with polypropylene mulch and decomposition of soil amendment restrict the gaseous exchange between the soil and atmosphere, thus creating anaerobic conditions and release of toxic substances such as acetic acid, butyric acid, and other volatiles which can be lethal to the soil-borne pathogens [114].

## 10. USE OF BOTANICALS

Mahadevan [115] reported that the presence of antifungal components in higher plants to have been recognized as an important factor for disease resistance due to their biodegradability and selective ability in their toxicity. They are considered as highly valuable in controlling some plant diseases [115]. Neem extract as one of the botanicals used in managing plant diseases has been reported to show antimicrobial activity with notable effects on *Sphaerotheca fuliginea* causing powdery mildew [116]. Significant levels of control of Fusarium wilt of tomato [117], downy mildew of grape caused by *Plasmophora viticola*, late leaf spot incited by *Phaeoisariopsis personata* and rust of ground nut caused by *Puccinia arachidis* has been documented [118]. Tests done by Kimaru et al

[117] revealed that the neem cake powder contained ingredients that had fungistatic effects against *Fusarium* wilt of tomatoes. Qasem and Abu-Blan [119] studied the antifungal effect of aqueous extract of *Ranunculus* sp. against *F. oxysporum* f.sp. *lycopersici* *in vitro* and observed that there was a strong inhibition in the mycelial growth of the pathogen. In another study, Shivpuri et al. [120] reported the toxicity of ethanol leaf extracts of neem and *Datura* against *F. oxysporum* and *R. solani* under laboratory conditions.

### 11. USE OF RESISTANT OR TOLERANT VARIETIES

Use of resistant or tolerant varieties is the most eco-friendly and cost-effective disease management practice [121,122] (2011). Cerkauskas [123] reported the availability of varieties that are resistant to races I and II of *Fusarium oxysporum* f. sp. *lycopersici* which will offer a valid alternative to the use of chemicals. However, additional pathogenic strain (Race 3) of the pathogen which affects the developed resistant varieties has been reported in several countries [22,124,125,126]. For this reason, complementary methods of controlling the disease need to be explored.

### 12. GOOD AGRICULTURAL PRACTICES (GAP)

Improvement of plant vigour through good farm management often helps in increasing resistance to pathogen attack. Earlier reports ascertained that altering of the soil pH by appropriate choice of fertilizer led to amelioration of the environment making it unfavourable for disease development [127]. "Raising soil pH between 6 - 7.5 and fertilizing with nitrogenous fertilizer controlled *Fusarium* wilt of tomato and chrysanthemum" [128]. Crop rotation with non-solanaceous crops such as cereals helped in reducing *Fusarium* wilt [129]. Use of soil amendments has been reported to be more promising than fungicides in controlling *Fusarium* wilt because it led to the reduction or elimination of inoculum of the pathogen in the soil [130] Jeff [131] and Kimaru et al. [117] attributed "the effectiveness of soil amendment against soil-borne pathogen to enhanced host nutrition, proliferation of antagonistic microorganisms and heat produced during decomposition". "Decomposed organic materials have long been recommended in most agricultural and horticultural systems using organic farming methods as they offered possible control strategies for soil-borne diseases" [117].

### 13. INTEGRATED DISEASE MANAGEMENT (IDM)

Waiganjo et al. [132] recognized "Integrated Pest Management (IPM) as effective approach for increasing agricultural productivity and combating environmental degradation in developing countries. The Integrated Pest Management seeks to improve the productivity of high-value marketed horticultural crops in Africa. Practices that can help to build healthy soils include crop rotation, organic matter additions or using high-residue tillage implements". "A significant amount of research has been conducted on the suppression of pests and diseases through the application of compost products worldwide. The results have shown that composts can provide natural biological control of soil-borne diseases affecting collar and roots as well as plant foliage" [133]. "The inclusion of green manures and cover crops in a rotation is an excellent way to improve soil fertility, suppress weeds and provide a break in pest cycles" [131]. A research conducted by Singh et al. [134] disclosed effective suppression of *Fusarium* wilt of tomato by integrating plant extracts with biocontrol agents and chemicals which significantly suppressed *Fusarium oxysporum* f. sp. *lycopersici*.

### 14. CONCLUSION

Chemical fungicides application are often not effective enough to control *Fusarium* wilts and other soil-borne diseases, and recently there is global outcry and public concerns about their deleterious effects. This necessitates the search for viable alternatives which are safe and eco-friendly. This review has explained the non-chemical methods that could conceivably be exploited for effective management of FOL. It also attributed difficulties in the control of FOL to the emergence of new pathogenic races, elimination of bio-control organisms as a result of indiscriminate use of fungicides and inadequate land for long-term crop rotation. Considering the adverse effects of chemicals to the environment and human health, it is therefore, necessary to find novel alternatives to control *Fusarium* wilt of tomato. Adoption of these methods for *Fusarium* wilt management and other soil-borne disease on tomatoes and vegetables may not eradicate all the pathogenic fungi from the soil. The use of integrated disease management strategies may offer solution to this where some of the eco-friendly ways outlined may be applied with/or without judicious application of fungicides.



Therefore, the use of integrated approach is highly recommended to achieve sustainable tomato production.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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