

More insights into approaches with potential effect against pathogenic *Fusarium* species

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Abstract

Fusarium species are a group of pathogenic fungi responsible for different plant diseases as well as damage of stored products like banana, rice and cereals. They are characterized by a widespread distribution because of their capacity to grow in different substrates. Against these pathogenic fungi, many approaches have been tried to protect crops and stored products from their devastating effects. Chemical molecules are widely used for their effective activity as fungicides, but they may cause important damage to crops, human and environment. For this reason, researchers are trying to find an alternative allowing the farmer to avoid these negative effects of chemical fungicides. Among these potential alternatives, we find the use of natural products (monoterpenes, flavonoids etc), plant extracts (including essential oils), biological control agents (bacteria and fungi), antimicrobial nanomaterials and approaches aiming to improve plant resistance and defence.

Keywords: Control, Disease, *Fusarium*, Resistance, Treatment.

Introduction

Fusarium is a fungal genus belonging to the family of Nectriaceae. Species of this genus are hyaline filamentous fungi characterized by their cosmopolitan distribution. They can be found in soil, in plant roots and aerial parts, in plant debris and other organic substrates (Nelson *et al.*, 1994; Lombard *et al.*, 2015). Their capacity to grow in many different substrates and the fact that they have developed excellent mechanisms for dispersal may explain their widespread distribution. *Fusarium* species are known to cause many plant diseases like crown rot, head blight, scab on cereal grains, vascular wilts on a wide range of horticultural crops; root rots, cankers and other diseases such as pokkah-boeng on sugarcane and bakanae disease of rice (Asam *et al.*, 2017). *Fusarium* species also cause many human and animal diseases because of the mycotoxins they can produce such as fumonisins, trichothecenes and zearalenone (Nesvorna *et al.* 2012; Broyde and Dore, 2013). In addition, species of *Fusarium* can be responsible for damage of stored products like cereals, rice and many other crops (Nesvorna *et al.*, 2012).

One of the most pathogenic fungus devastating plants is *Fusarium oxysporum* f. sp. *albidenis*. It is responsible for vascular wilt of date palm (*Phoenix dactylifera*) known under the name of Bayoud disease. The Bayoud is the most important disease of date palm in Morocco and around the world. It has destroyed about 60% of Moroccan palm plantations causing economical, ecological and social damages (El Modafar, 2010). It attacks the plant through roots and then spreads in other parts of

the tree by vascular system leading to foliar withering and death of palm trees (El Modafar, 2010; Saleh *et al.*, 2017). Its transmission and surviving under adverse environmental conditions are related to its typical micro and macroconidia, as well as chlamydospores it can produce. The releases of propagules ensure dissemination of the fungus through infested soil, irrigation water and infected date palm tissues. Chemical, prophylactics, genetics and biological controls have been tried in order to reduce the dissemination of this fungal disease but without hoped results (Momma *et al.*, 2010). *Fusarium solani* is responsible for wilt and rot diseases in different tree species including *Erythrina suberosa* and *Delbergia sissoo* (Javaid *et al.*, 2005; Bajwa *et al.*, 2003 a, b).

Fusarium moniliforme is an endophytic fungus attacking maize by the production of mycotoxin called fumonisin (FB1 and FB2). Clonal infection of seeds and plant debris are the means of the fungal transmission (Ketabchi and Shahrtaq, 2011). Likewise, *Fusarium thapsinum* causes stalk rot in maize (Tahir *et al.*, 2018). Tomato is among plants affected by *Fusarium oxysporum* f. sp. *lycopersici* (Khurshid *et al.*, 2014, 2016). It causes one of the most damaging tomato diseases leading to important losses (Ojha and Chatterjee, 2012). This fungus can infest tomato crops via contaminated seeds (Reis and Boiteux, 2007). Sweet potato is also a plant infected by *Fusarium oxysporum* f. sp. *batatas*. This fungal infection of sweet potato is characterized essentially by wilting and leaf yellowing. Browning of vascular tissues in the lower stems is also observed in infected plants (Thompson *et al.*, 2011). *Fusarium* wilt is a damaging disease of

cotton responsible for important losses in cotton-producing countries like Australia, USA, Egypt, Tanzania and China (Abd-El Salam *et al.*, 2014). This disease is caused by *F. oxysporum* f. sp. *vasinfectum* that reduces cotton production by about 100,000 bales per year in the USA (Davis *et al.*, 2006). Another devastating fungal infection is banana fusariosis caused by *F. oxysporum* f. sp. *cubense*. This disease is responsible for significant losses in countries like Ivory coast, is characterized by a uniform and deadly yellowing of banana leaves (Kobert *et al.*, 2017). Lentil also can be attacked by a *Fusarium* species called *F. oxysporum* f. sp. *lentis* causing a disease characterized by stunting, wilting and reduction of root system. It has been reported also that internal vascular discoloration of the lower stem is an important symptom observed in infected lentil (Hiremani and Dubey, 2019). In the summer of 1999-2000, a severe loss of lentil seedlings contaminated by this fungus occurred in Italy (Tosi and Cappeli, 2001).

Many other damaging plant diseases are caused by *Fusarium* species. Sugarcane wilt by *Fusarium sacchari*, Bakanae in rice by *Fusarium fujikuroi*, flowering malformation of mango by *Fusarium manginifera*, green point gall of cacao by *Fusarium decemcellulare*, oil palm wilt by *F. oxysporum* f. sp. *elaedis*, crown rot by *Fusarium pallidoroseum*, Panama disease by *F. oxysporum* f. sp. *cubensis*, Gibberella zea by *Fusarium graminearum*, Papaya internal fruit rot by *Fusarium* spp., pitch caner in pine by *Fusarium circinatum*, Corm rot of gladiolus by *F. oxysporum* f. sp. *gladioli*, basal rot of onion caused by *F. oxysporum* f. sp. *cepae* and *Fusarium* wilt of *Washingtonia robusta* by *F. oxysporum* f. sp. *palmarum* (Khan *et al.*, 2010; Riaz *et al.*, 2010; Giesbrech *et al.*, 2013; Javaid and Akhtar, 2015; Slinski *et al.*, 2015; Omar *et al.*, 2018; Million *et al.*, 2019; Scherm *et al.*, 2019).

The aim of this review is to present several approaches with a potential effect against some plant diseases caused by different *Fusarium* species. The species of *Fusarium* genus have a lot of common points related to their structure, physiology and mode of infection. Therefore, when an approach, or product, shows an effect on a specific species, it could be interesting to test it against another species. The approaches discussed in this review are related to treatment by natural and synthesized molecules, treatment by plant extracts and essential oils, biological control, nanoparticles and methods used to enhance plant resistance.

Use of natural products

Natural products, extracted from plants or microorganisms, are widely used for their diverse biological properties such as antibacterial, antifungal, herbicidal, anticancer and antioxidant activities (Khan and Javaid, 2019, 2020; Javaid *et*

al., 2020; Javed *et al.*, 2021). It has been shown in many studies that microorganisms and plants are able to produce chemical molecules or hormones that they use for their defence against other microorganisms. Sometimes, these molecules are used as template to synthesize analogues having the same activities (Ntie-Kang, 2016; Sunil and Hoskins, 2017). Many classes of natural compounds have been studied for their potential effect against *Fusarium* species.

Monoterpenes: Monoterpenes, classified into monoterpene hydrocarbons and oxygenated monoterpenes, are the main constituents of the majority of plant essential oils. It has been reported that this class possess an important insecticidal, herbicidal, fungicidal and bactericidal properties (Caravon *et al.*, 2014; Karlsson *et al.*, 2017; Ghasemi *et al.*, 2020). Twelve monoterpenes namely camphene, camphor, carvone, 1,8-cineole, cuminaldehyde, fenchone, geraniol, limonene, linalool, menthol, myrcene and thymol were tested against four plant pathogenic fungi including *F. oxysporum*. The study was performed using mycelial growth inhibitory technique. Results reported that thymol, (S)-limonene and 1,8-cineole have antifungal effect on the tested fungi, and thymol was the most active against *F. oxysporum* with EC₅₀ of 50.35 mg mL⁻¹. The same study reported that thymol acted by inhibiting pectin methyl esterase and cellulase of tested fungi (Marei *et al.*, 2012).

Flavonoids: Flavonoids, secondary metabolites generally located in plant leaves, are not only a constitutive agents but they also play an important role in plant defence against microbes (Kanwal *et al.*, 2010; Mathesius, 2018; Laila, 2020; Biliska *et al.*, 2018). Several flavonoids glycosides isolated from carnation (*Dianthus caryophyllus*) were found antifungal against different pathotypes of *F. oxysporum* f. sp. *dianthi* (Galeotti *et al.*, 2008). Extract of barley were tested against *Fusarium poae*, *F. culmorum* and *F. graminearum*, and the result revealed that the flavonoid dihydroquercetin was a strong inhibitor of *Fusarium* growth and macrospore formation (Skadhauge *et al.*, 1997; Bollina *et al.*, 2011). *In vitro* effect of an extract enriched in flavonoids obtained from *Triguero asparagus*, was checked on *Fusarium* species pathogenic to asparagus, carnation and strawberry. A significant inhibition of mycelial growth and sporulation of *F. oxysporum* f. sp. *asparagi*, *F. oxysporum* f. sp. *dianthi* and *Fusarium* sp. pathogenic to strawberry was observed without affecting plant safety (Rozado-Alvares *et al.*, 2014). Strains of *Fusarium culmorum* and *F. graminearum* were incubated *in vitro* with flavonoids which are usually produced by cereals in response to fungal infections. Results showed that mycotoxin production by the two fungi was significantly reduced by luteolin, kaempferol, naringenin and apigenin. The same study also

established a relationship between antifungal effect of flavonoids and their antioxidant and antiradical properties (Bilska *et al.*, 2018).

Alkaloids: An alkaloid extracted from *Prosopis juliflora* (weed plant) was evaluated for its antifungal effect on toxigenic and non-toxicogenic *Fusarium verticillioides* isolated from seeds of paddy, maize and sorghum. Compared to some conventional fungicides (copper oxychloride, thiophanate-methyl, chlorothalonil and carbendazim), it has been shown that this natural alkaloid was the most effective at low concentration (300 $\mu\text{g mL}^{-1}$) than those chemical fungicides which were effective only at high concentrations (2000 $\mu\text{g mL}^{-1}$) (Deepa *et al.*, 2012). Some new saturated pyrrolizidine monoester alkaloids, 3-acetyltrachelanthamine, floridine, floridinone and floridimine, heliovicine were isolated from *Heliotropium joridum* and their biological activity revealed that 3-acetyltrachelanthamine and floridine have the most important effect against *Fusarium moniliforme* (Reina *et al.*, 1997). Besides monoterpenes, flavonoids and alkaloids, it has been reported in the literature that other classes of natural products such as polyphenols, tanins, saponins and sterols have a significant antifungal effect against some pathogenic *Fusarium* species (Rodriguez *et al.*, 2012; Isidore *et al.*, 2018).

Eugenol: It has been shown that small concentrations of eugenol, pure product extracted from clove, is able to induce the destruction of structure of *Fusarium oxysporum* f. sp. *vasinfectum* (Abd-Assalam and Khokhlov, 2015). Eugenol also showed toxicity against other pathogenic fungi (Morcia *et al.*, 2012; Matan *et al.*, 2014). Because of its lipophilic character, eugenol pass easily through cell membrane (Bakkali *et al.*, 2008). Its mechanism of action may be related to the inactivation of enzymes, altering fluidity and permeability of cell membrane or disturb the genetic material (Braga *et al.*, 2007; Woo *et al.*, 2009). Mechanism of action of eugenol can also involve ergosterol biosynthesis inhibition (de Oliveira *et al.*, 2013).

Alphapinene and betacaryophyllene: These are the major components of *Chromolaena odorata* leaf extracts. These two molecules exhibited antifungal activities against *Fusarium* species and several other fungi (Ling *et al.*, 2003; Noudogbessi *et al.*, 2008).

Use of synthesized molecules

In addition to natural molecules, synthesized molecules, with less negative effect on crops and environment, are also studied for their potential activity on pathogenic *Fusarium* species. Diphenyl diselenide and some of its synthetic analogues were evaluated *in vitro* for their antifungal activity against 116 strains of pathogenic fungi, including *Fusarium* species. The compounds showed antifungal activity against some pathogenic *Fusarium* species with inhibitory activity of 2–16 $\mu\text{g mL}^{-1}$ and minimum

fungicidal concentration of 4–64 $\mu\text{g mL}^{-1}$ (Loreto *et al.*, 2011). In another study, some benzimidazoles, aromatic hydrocarbons and sterol biosynthesis inhibitors showed a significant activity to prevent or control pathogenic *Fusarium* spp. growth (Daferera *et al.*, 2003). Furthermore, five substituted flavones and their mixtures with unsubstituted flavone or flavanone were evaluated for their antifungal activity against five *Fusarium* spp. Among these molecules, 4'-methylflavone and 2',6'-dichloroflavone showed the most important effect. The difference observed in the molecules activity were found related to their chemical structure (Silva *et al.*, 1998).

Use of essential oils and plant extracts

There is an increasing interest in the use of essential oils for their biological activity (Ferdosi *et al.*, 2020, 2021). Because of their safty, they are considered as an alternative to chemical agents which are toxic for both plants, human and environment (Tepe *et al.*, 2005; Srenivasa *et al.*, 2011; Naz *et al.*, 2014).

In an interesting study, essential oils of clove (*Syzygium aromaticum*), lemongrass (*Cymbopogon citratus*), mint (*Mentha piperita*) and eucalyptus (*Eucalyptus globulus*) were tested against *F. oxysporum* f. sp. *lycopersici* 1322. All the tested essential oils showed an inhibitory effect on the fungal growth in dose-dependent way. The most active was clove essential oil showing complete inhibition of mycelial growth (MIC = 31.25 ppm and IC₅₀ = 18.2) and inhibition of spore germination (0.3 ppm). The other essential oils (lemongrass, mint and eucalyptus) were also active but at higher concentrations. GC-MS analysis showed that the major components of clove essential oil were eugenol (75.41%), E-caryophyllene (15.11%) and α -humulene (3.78%). Results of the same study, using scanning electron microscopy and atomic force microscopy, showed shrivelled hyphae and disrupted spores in clove essential oil treated samples. Taken together, these results suggested that clove essential oil could be used as biofungicide for the control of *F. oxysporum* f. sp. *lycopersici* (Sharma *et al.*, 2017). In another study, essential oils of many plants like eucalyptus, clove, cedar wood, citronella and neem showed antifungal activities against nine *Fusarium* species at concentrations of 500–2500 ppm. Among the tested essential oils, that of citronella had the highest inhibitory effect against *F. oxysporum* (Sreenivasa *et al.*, 2011). In Brazil, important losses in *Piper nigrum* cultivation are caused by *F. solani* f. sp. *piperis*. Essential oil of *Piper divaricatum*, with methyleugenol (75%) and eugenol (10%) as major constituents, was tested at concentrations of 0.25 to 2.5 mg mL^{-1} against this fungus. Results reported that the essential oil had a strong antifungal index (18% to 100%). The same study, using 3D structure of the β -glucosidase from *Fusarium solani* f. sp. *piperis*, showed that β -glucosidase residues were

strongly involved in protein-engenol/methyleugenol interaction, explaining the antifungal action of these two phenylpropenes against the pathogenic fungus (Da Silva, 2014).

Fusarium culmorum and *Fusarium verticillioides* are two fungi of stored rice mycobiota. Essential oils of bay leaf, cinnamon, clove and oregano were tested against these fungi, *in vitro* and *in vivo*, and they showed significant control of fungal growth. In the *in vitro* test, oregano essential oil was the most effective (100% inhibiting growth). The other essential oils (bay leaf, cinnamon, clove) also showed marked effect as they reduced fungal growth by 90%. GC-MS analysis of these essential oils revealed that they are rich in oxygenated molecules: 92% in cinnamon (eugenol 60% and eugenyl acetate 18.3%), 90.3% in clove (eugenol 89.8%), 78.8% in bay leaf (eucalyptol 51%) and 71.8% in oregano (carvacrol 49.6% and thymol 21.2%). The content of monoterpenes and sesquiterpenes were 18%, 9%, 5% and 25% in bay leaf, clove, cinnamon and oregano, respectively (Rosello *et al.*, 2015). Essential oil of mexican oregano (*Lippia berlandieri*) was found a potential inhibitor of *F. oxysporum* with MIC of 0.15 $\mu\text{L mL}^{-1}$ and totally inhibited the fungal biomass production at 0.2 $\mu\text{L mL}^{-1}$. When tested on infected tomato seeds, oregano essential oil at 0.5% completely inhibits the fungus proliferation without affecting seed germination rates (Cueto-Wong *et al.*, 2010). Essential oils of origanum (*Origanum onites*), lavender (*Lavandula stoechas* subsp. *stoechas*), fennel (*Foeniculum vulgare*), laurel (*Laurus nobilis* L.) and myrtle (*Myrtus communis*) markedly suppressed mycelial growth and spore germination of *F. oxysporum* f. sp. *radicis-cucumerinum* causing root and stem rot of cucumber (Soylu and Incecara, 2017). Essential oils of *Rosmarinus officinalis* and *Salvia officinalis* showed pronounced effect against pathogenic *Fusarium* species (Mekonnen *et al.*, 2019).

Plant extracts prepared in different solvents (ethanol, acetone, methanol, ethyle acetat etc) have been studied for various biological activities. Ethanolic and aqueous extracts from leaves of six plants were evaluated for their antifungal activity against *Fusarium* sp. The study reported that ethanolic extracts were more effectives than aqueous ones. Chemical analysis revealed that polyphenols, flavonoids, polyterpenes, sterols and tannins could be responsible for antifungal activity of these ethanolic extracts (Isidore *et al.*, 2018). Aqueous and alcoholic extracts of *Acacia farnesiana* were tested against *F. oxysporum* f. sp. *lycopersici*. Results showed an important percentage of inhibition of mycelial growth (> 90% after 72 h). This antifungal effect was related to flavonoids, tannins, phenols, alkaloids and saponins present in the extracts (Rodrigez Pedroso *et al.*, 2012). Furthermore, aqueous and different solvent extracts from *Prosopis juliflora* (weed plant) had a significant fungitoxicity

against all the strains of toxigenic and non-toxigenic *Fusarium verticillioides* isolated from seeds of paddy, maize and sorghum (Deepa *et al.*, 2012). Other plant extracts like *Chromolaena odorata* showed marked activity against *Fusarium* species (Kra *et al.*, 2009). It has been shown that aqueous extracts from fresh aerial parts of four plant species (*Artemisia herba-alba*, *Cotula cinerea*, *Asphodelus tenuifolius* and *Euphorbia guyoniana*) could inhibit *Fusarium graminearum* and *Fusarium sporotrichioides* growth. The activity of these extracts was related to their tannins, flavonoids, saponins, steroids and alkaloids (Salhi *et al.*, 2017).

In a study, 131 extracts, resins, oils and mother tinctures from 63 plants were evaluated for their potential activity against *Fusarium guttiforme* using plate-hole method. On the other hand, mother tinctures were tested for their capacity to control pineapple fusariosis *in situ*. Results showed that mother tinctures of *Glycyrrhiza glabra*, *Myroxylon balsamum*, *Aloe vera* and *Allium sativum*, resin of *Protium heptaphyllum* and crude extracts of *Rhizophora mangle*, exhibited significant antifungal effects against *Fusarium guttiforme* (Costa *et al.*, 2016). Several plant extracts were evaluated for their effect on soil infested with *F. oxysporum* f. sp. *chrysanthemi*. The results showed that aqueous emulsions (10%) of pepper/mustard, cassia and clove extracts reduced the fungal population density by 99.9, 96.1 and 97.5%, respectively, after 3 days (Bowers and Locke, 2000). Khurshid *et al.* (2018) reported that methanolic extract of aerial parts of an allelopathic grass *Cenchrus pennisetiformis* and its various organic solvent fractions showed pronounced antifungal activity against *F. oxysporum* f. sp. *lycopersici*. They further reported that soil amendment with dry biomass of this grass significantly reduced disease incidence in tomato plants. Likewise, methanolic extract of *Coronopus didymus* significantly inhibited the growth of *Fusarium moniliforme* (Javaid *et al.*, 2018). Aftab *et al.* (2019) reported that methanolic extract of vegetative parts of *Nigella sativa* can significantly suppress the growth of *F. oxysporum*. Shoot extracts of *Sisymbrium irio* contain potent antifungal compounds namely, β -sitosterol and fatty acid methyl esters against *Fusarium oxysporum* f. sp. *cepae* (Akhtar *et al.*, 2020). Similarly, leaf extract of *Chenopodium murale* contains many antifungal compounds for the control of *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *cepae* (Naqvi *et al.*, 2019; Nawaz *et al.*, 2020).

Biological control of *Fusarium* spp.

Intensive application of chemical antifungal has lead to an increased negative impacts on plants, human, soil flora and environment. Therefore, biological control could be an excellent alternative of the chemical treatment. Biological control of fungal diseases is based on the use of microorganisms to

fight pathogenic fungi responsible for diseases. Against *Fusarium* species, many bacteria and fungi were tested. Rhizobacteria, especially actinomycetes and *Pseudomonas* sp, are among the microorganisms mostly used in the field of biological control.

Actinomycètes: Rhizospheric actinomycetes represent excellent candidates in biological control of fungal diseases. In fact, 60% of natural antifungals used in agriculture are derived from the actinomycete genus *Sytreptomyces* (Bubici, 2018). Because of their abundance in the natural soil and plant roots, they are frequently used as biological control agents (Aichour *et al.*, 2012). Several biocontrol trials using Streptomycetes were conducted against four pathogenic *Fusarium* species namely *F. fujikuroi*, *F. graminearum*, *F. solani* and *F. oxysporum*. Results showed marked *Fusarium* wilts reduction of 0–50%. Yekkour *et al.* (2012) reported the capability of the 133 strains of filamentous actinomycetes to inhibit growth of different *Fusarium* species (Yekkour *et al.*, 2012). In a study carried out under field conditions, cucumber wilt caused by *F. oxysporum* f. sp. *cucumerinum* was treated by a suspension of *Streptomyces albospinus* CT205 spore. It has been shown that this treatment reduced pathogenic effect by 22% and plant death by 33%. Also, the numbers of *Fusarium* colony forming units was significantly decreased and the microbial structure in rhizosphere was improved (Wang *et al.*, 2016). *Actinomadura* strain AC170 isolated from saharian palm was evaluated for its antifungal activity against several pathogenic and toxigenic fungi. Results showed that butanolic extract of this bacteria had an important antifungal effect against the all tested fungal strains. This activity was related to an aromatic compound present in this extract (Badji *et al.*, 2005). It has been reported in other study that five strains of *Streptomyces* spp. (CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90) isolated from herbal vermicomposts showed remarkable control effects against *F. oxysporum* f. sp. *ciceris* (Gopalakrishnan *et al.*, 2011). The biocontrol mechanisms of *Streptomyces* spp. would be assured by their chitinolytic activity, endophytic trait, volatil organic molecules production and plant resistance induction (Bubici, 2018).

Pseudomonas: *Pseudomonas* is a genus of gram-negative bacteria from Pseudomonadaceae family that counts about 191 described species (Meghdas *et al.*, 2004). This genus is among the bacteria mostly used in biological control of plants diseases (Sharf *et al.*, 2021). Tested against some fungi including *F. oxysporum*, several strains of *Pseudomonas* showed an important growth inhibition effect (Sindhu *et al.*, 1999). These bacteria are excellent competitors vis à vis fungal and bacterial microflora in the soil. They have a short generation time *in situ* and are able to use plants exudates as nutrients and chelate iron ions by the production of siderophores like pyoverdine (Aichour *et al.*, 2012). *Pseudomonas* effect is due to

the production of many secondary metabolites like phenazines, phloroglucinols, pyoluteorine, pyrrolnitrin, lipopeptides and hydrogen cyanure. *Pseudomonas fluorescens* is considered as the most important species of this genus because of its capability to produce different plants growth factors and bioactive molecules necessary to plant protection (Aichour *et al.*, 2012).

Bacillus: *Bacillus* is another genus of gram-positive bacteria genus with interesting properties as agent of biological control of fungal diseases. Two species of this genus have been studied in this sense. *Bacillus cereus* is responsible of foodborne intoxications. It has been reported that methanol extract of *Bacillus cereus* X16 inhibits the mycelial growth of *Fusarium roseum* var. *sambucinum*, *in vitro* and *in vivo*. The extract, made in the interaction region between fungal and host, contains many antifungal molecules (Sadfi *et al.*, 2002). *Bacillus subtilis* is an endophytic bacterium with an important effect in biological control of *Fusarium moniliforme*. This bacteria can reduce mycotoxin accumulation during the fungus growth phase. This bacterium is interesting since it is considered as an ecological homologue to *F. moniliforme* because it occupies the identical ecological niche within the plant (Bacon *et al.*, 2001).

Other bacterial isolates: Date palm is being destroyed by bayoud disease caused by *F. oxysporum* f. sp. *albedinis*. Isolates belonging to 21 bacteria including *Bacillus* spp., *Rhizobium* spp., *Ulocladium atrum*, *Candida guilliermondii*, *Pseudomonas* sp., *Rahnella aquatilis* and other bacteria, were evaluated for their effects on the mycelial growth and the sporulation of this pathogenic fungus. Results showed that *B. pumilus* W1, *R. aquatilis* W2 and *B. cereus* X16 inhibited the mycelial growth and sporulation of *F. oxysporum* f. sp. *albedinis* by 70–77% and 80–95%, respectively (El Hassni *et al.*, 2007).

Endophytic and other biocontrol fungi: Beside bacteria, some fungi, in particular endophytic fungi, have shown an excellent effect as agent of biological control against plants diseases. In a recent study, 30 endophytic fungal species have been isolated from different Chinese plants and evaluated for their effect against *F. oxysporum* f. sp. *cucumerinum*, causing wilt in cucumber. The test was performed using *in vitro* dual culture assay. The results reported that the 30 endophytic fungal isolates have important inhibiting effects on the mycelial colony growth of *F. oxysporum* f. sp. *cucumerinum* (> 66%) compared to control. The most effective isolates were those of *Penicillium* sp., *Guignardia mangiferae*, *Hypocrea* sp., *Neurospora* sp., *Eupenicillium javanicum*, and *Lasiodiplodia theobromae*. Greenhouse test showed that the most effective fungi suppressed wilt severity when co-inoculation with pathogen *F. oxysporum* f. sp. *cucumerinum*. Furthermore, the same study reported that these isolates increased plant height,

aerial fresh and dry weight of host plant (Abro *et al.*, 2019). Other study showed that species of genus *Trichoderma*, belonging to Hypocreaceae family, can be used as antagonistic in biological control of several soil-borne pathogens (Hanson and Howell 2004; Bajwa *et al.*, 2004). Five species of *Trichoderma* were tested *in vitro* for their antagonist effect against *F. oxysporum* causing wilt disease in sweet peppers. The results showed that *T. viride* had the best effect followed by *T. harzianum*, *T. aureoviride*, *T. koningii* and *T. pseudokoningii*. These species reduced colony growth of pathogenic fungus by 62, 36, 24, 18 and 6%, respectively (Sahi and Khalid, 2007). *Trichoderma* species also have potential to control postharvest growth and toxin production by *Fusarium moniliforme* (Bacon *et al.* 2001). There are also reports of control of *Fusarium solani*, *F. oxysporum* f. sp. *Cepae* and *F. oxysporum* f. sp. *lycopersici* by *Trichoderma harzianum* (Mukhtar *et al.*, 2006; Javaid *et al.*, 2014; Akhtar and Javaid, 2018)

Use of nanomaterials for control of *Fusarium* spp.

Efficient and ecosafe techniques to administrate insecticides, herbicides, fungicides and fertilizers are needed in order to protect consumers, plants and environment (Li *et al.*, 2007; Gogoi *et al.*, 2009). Biobased nanomaterials is one of these promising methods which could improve protocols to fight against plant diseases (Abd-Elsalam and Khokhlov, 2015; Um-e-Aiman *et al.*, 2021). It has been reported that nanobiocid, composed by biobased chemicals, are able to eliminate the causal agent of rice blast disease (Gogoi *et al.*, 2009).

Regarding *Fusarium* genus, different concentrations of eugenol oil nanoemulsions (EON) were tested against *F. oxysporum* f. sp. *vasinfectum* both *in vitro* and *in vivo*. Analysis of total proteins isolated from the fungus before and after treatment with EON was performed by SDS-PAGE. The results indicated that there was a decrease in extracellular soluble small molecular proteins. Also, light micrographs showed mycelia and spores treated with EON indicating the destruction of fungal structure (Abd-Assalam and Khokhlov, 2015). Toxicity of EON molecules on cotton plants was evaluated by studying viability on 10 cotton genotypes. It has been shown that only high concentration (10%) of emulsion, when used for *Fusarium* control in muskmelon, caused stunting and other phytotoxic symptoms (Morcia *et al.*, 2012; Bowers and Locke, 2000). Another study was carried out to evaluate the effect of silver nanoparticles (AgNPs) on *F. oxysporum*. Results showed that silver nanoparticles reduced colony formation of *F. oxysporum* in dose dependent manner. A concentration of 5000 ppm of AgNPs decreased colony formation to 35%, 27% and 24% after 1, 2 and 3 hours of exposure, respectively. Also, fungal colony formation is reduced by extended spore

exposure to silver nanoparticles (Abkhoo and Panjehkeh, 2017).

A study comparing chitosan (CS) and chitosan nanoparticles (CS/NPs) was conducted against *Fusarium graminearum* causing head blight on wheat. Technique used for CS/NPs preparation was based on the ionic gelation of CS with tripolyphosphate with centrifugation and pH modification. Different CS/NPs were prepared based on particle diameter and molecular weights. Results showed that fungal growth reduction, at concentrations of 1000 and 5000 ppm, was 68.18% and 77.5% for CS and its NPs, respectively (Kheiri *et al.*, 2017). Taken together, these results suggest that CS and NPs can be used as agents of crop protection. In greenhouse trials, concerning disease progress at 28 days after inoculation (dpi), the area under curve was 7.36 and 7.7 for control plants treated with aqueous solution of acetic acid and distilled water, respectively. On the other hand, plants treated with CS and NPs had approximately 3.61 and 3.34, respectively (Kheiri *et al.*, 2017).

Approaches providing plant resistance against *Fusarium* spp.

Enhancing plant resistance is a promising approach to fight diseases. It is based on inducing systemic resistance by employing different techniques like localized pretreatment with elicitors (Hammerschmidt *et al.*, 2001) or application of microorganism isolates (El Hassni *et al.*, 2004). Molecules providing plant resistance may be secreted by microorganism membrane. It's the case of lipopolysaccharides (Reitz *et al.*, 2000), N-acetylchitooligosaccharides (Yamagushi *et al.*, 2000) or glycoproteins (Lepoivre and Semal, 1989). Also, inductor oligosaccharides may be produced by a membrane of host cells under bacterial or fungal enzymatic reaction (Lepoivre and Semal, 1993). Once in contact with plants, elicitors cause the expression of biochemical changes involved in defence responses induction. Such biochemical reactions include activation of various defence-related enzymes, accumulation of active oxygen species and synthesis of pathogenesis-related proteins and phytoalexins (Nurnberger and Lipka, 2005). Jasmonic acid (JA) is among elicitors which has been studied for its potential effect against *F. oxysporum* f. sp. *albedinis* causing bayoud (date palm disease). It is a natural phytohormone involved in plant development (Creelaman and Mullet, 1997). The aim of the study was to evaluate the effect of this elicitor on two enzymes peroxidases and polyphenoloxidases involved in plant defence. Results reported that the activity of the two enzymes *viz.* was increased by exogenous application of Jasmonic acid at 50 mM compared to untreated plants. Also, chromatographic analysis by PAGE showed an important band intensity related to the major peroxidases and polyphenoloxidases isoforms

in seedlings treated by both jasmonic acid and the fungus. Examination of plants showed a limited necrotic lesions in asymptomatic plants compared to symptomatic ones. All these results indicated that the elicitor jasmonic acid could enhance date palm resistance to this pathogen by a mechanism involving the activation of enzymes like peroxidases and polyphenoloxidases (Jaiti *et al.*, 2009). Another study trying to enhance date palm resistance against bayoud disease was conducted by El Hassni *et al.* (2004). It consisted in the pretreatment of date palm seedlings with an hypoaggressive *F. oxysporum* f. sp. *albedinis* isolate. Results showed that this isolate protected date palm against further infection by the pathogen. The high level of phenolics and peroxidase activity, involved in date palm resistance to *F. oxysporum* f. sp. *albedinis* indicate the ability of pretreated date palm to establish a faster defence response in its roots. Also, height level of non-constitutive hydroxycinnamic acid derivatives and constitutive caffeoylshikimic acids was observed in

pretreated plants. These constituents are known to play an important role in date palm defence against *F. oxysporum* f. sp. *albedinis* (El Hassni *et al.*, 2004). Another study of the same team reported that application of bacterial isolates into date palm seedlings improved its defence reactions. These reactions evaluated by non-constitutive hydroxycinnamic acid derivatives and sinapic derivative amounts. It has been shown, in the same study, that resistance reaction was more pronounced in resistant cultivar compared to the sensitive one (El Hassni *et al.*, 2007). *Fusarium oxysporum* f. sp. *lentis* MR84 is another *Fusarium* species responsible of lentil disease. It has been reported that culture filtrate of *Trichoderma harzianum* T-ADS protected totally lentil plants against infection by this pathogenic fungus without interaction between the two fungi, which suggests that *Trichoderma harzianum* T-ADS acted by enhancing plant resistance (Essalmani and Lahlou, 2004).

Table 1: Approaches with potential effect against *Fusarium* species.

Approach	Examples
Natural products	Monoterpenes: camphene, carvone, cineol, linalool... Flavonoids: by luteolin, kaempferol, naringenin and apigenin... Alkaloids: pyrrolizidine monoester alkaloids, 3'-acetyltrachelanthamine, flordidine, flordinine and flordimine, heliovicine... Eugenol Alphapinene and betacaryophylene
Synthesized molecules	Diphenyl diselenide Benzimidazoles Aromatic hydrocarbons Substituted flavones : 4'-methylflavone and 2',6'-dichloroflavone
Essential oils and plant extracts	Essential oils of clove (<i>Syzygium aromaticum</i>), lemongrass (<i>Cymbopogon citratus</i>), mint (<i>Mentha piperita</i>) and eucalyptus (<i>Eucalyptus globulus</i>) Aqueous extracts from fresh aerial parts of four plant species (<i>Artemisia herba-alba</i> , <i>Cotula cinerea</i> , <i>Asphodelus tenuifolius</i> and <i>Euphorbia guyoniana</i>) 131 extracts, resins, oils and mother tinctures from 63 plants
Biological control	Actinomycètes <i>Pseudomonas</i> <i>Bacillus</i> Endophytic and other biocontrol fungi
nanomaterials	Eugenol oil nanoemulsions Chitosan nanoparticles
Approachs providing plant resistance	lipopolysaccharids N-acétylchitoooligosaccharids – glycoproteins Jasmonic acid Bacterial isolates
Combination of different approaches	<i>Bacillus pumilus</i> + chitosan Botanical formulations + biocontrol agents

Combination of different approaches

Some researchers have tried to combine different biocontrol approaches to fight plants diseases. *Fusarium oxysporum* f. sp.

radicislycopersici is a vascular fungus that attacks tomato. *Bacillus pumilus* alone or in combination with chitosan was evaluated for their capacity to induce plant resistance against this pathogenic

fungus. Chitosan is a molecule extracted from chitin which is a natural fiber existing in shell of crustaceans and insects. The study was performed by light and transmission electron microscopy and gold cytochemistry. Ultrastructure analysis reported that in root tissues of plants treated only with bacteria (without chitosan), there was a limited development of the fungus and important changes in the host physiology compared to control. On the other hand, an increase in the cellular changes extent and magnitude was observed when chitosan was associated with bacteria. The same study showed that abnormal deposit of chitin-enriched (typical fungal cell reaction) was observed only when the plants were grown in the presence of both *Bacillus pumilus* and chitosan (Benhammou *et al.*, 1998). Akila *et al.* (2011) reported that combination of botanical formulations and biocontrol agents can have an important effect in the control of pathogenic fungi. Two botanical fungicides (Wanis 20 EC and Damet 50 EC) and two bacteria (*Pseudomonas*

fluorescens 1, Pfl and *Bacillus subtilis*, TRC 54) were tested individually and in combination against *F. oxysporum* f. sp. *ubense* causing banana wilt. The results of his study, conducted in greenhouse and in field conditions, showed complete inhibition of mycelial growth of the pathogen. The effect of this combination was related to the production of peroxidase and polyphenol oxidase involved in plant defence.

Conclusion

Because of their devastating effect as plant pathogenic fungi, *Fusarium* species are a subject of many studies where researchers tried to find means to fight them. These studies are, in general, focusing on the use of natural products, plant extracts, biological control using potential agents like bacteria and fungi and approaches aiming to enhance plant resistance. Nanoparticles are other sophisticated materials studied in this sense.

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