MANAGEMENT TACTICS TOWARDS RED ROT; CANCER OF SUGARCAN –A review

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ABSTRACT

Red rot of sugarcane, the most ravaging biotic stress to sugarcane and is responsible for the withdrawal of various varieties in sugarcane cultivation across the globe. The etiological agent of red rot disease (*Colletotrichum falcatum)* significantly degrades the quality and productivity of sugarcane. This disease causes 5-50% economic loss in sugarcane production due to reduced sugar recovery and cane weight by 31-75% and 29-83%, respectively. Substantial work has been done on pathogenic variability, screening, epidemiology, pathogenicity determinants, and disease resistance mechanism. It seems that there is a dearth of information on early detection and strategies to control pathogen because of many overlapping characteristics within the species complex. Various strategies such as cultural control, chemical control and biological control have been introduced to show promising results to combat this Phyto pathological challenge. The objective of the present manuscript is to summarize pathogen profile, symptomology, epidemiology, strategies and some future directions for diseases management. The present review is expected to be fruitful for further investigations.

Key words: *Saccharum officinarum*, *Colletotrichum falcatum*, Chemicals, Nanoparticles, Phytoextracts, Biological control

INTRODUCTION

Sugarcane (*Saccharum officinarum*), is a significant cash crop which is grown in sub-tropical and tropical regions of the world. It is a perennial crop of the genus Saccharum. Major sugarcane growing countries are Brazil, India, Thailand, Pakistan, China, Mexico, the United States of America, and Australia (FAO 2019). Approximately 1.91 billion tonnes of sugarcane was produced throughout the world, half of which is harvested from India and Brazil only (Factfish 2015) while in Pakistan, sugarcane covers an area of 1.17 million ha with production of 62.6 million tonnes (GOP 2014). It is cultivated in all the provinces (Punjab, Sindh, KPK and Balochistan) of Pakistan and its total production in Punjab (57,152), Sindh (19,800), KPK (6,150) and Balochistan (48) million tones (USDA 2021) which is also shown in Fig 1. It is a rich source of sugar ($C_{12}H_{22}O_{11}$) which accounts for approximately 2/3rd of sugar production globally (Menossi et al. 2008). A number of byproducts are prepared from sugarcane like press mud, molasses, and bagasse. Press mud provides a significant amount of organic manure which acts as ameliorates and is used as a substrate in bio-composting (Razzaq 2008; Bokhtiar et al. 2001; Chand et al. 2011) while molasses, is used for the preparation of foodstuff, animal feeds, and for the generation of alcohol (C_2H_5OH) and bagasse is used to make fuel, cardboard, and paper (Sardar et al. 2013). Beside these, it is a potential source for the production of bioenergy (Gianotto et al. 2011).

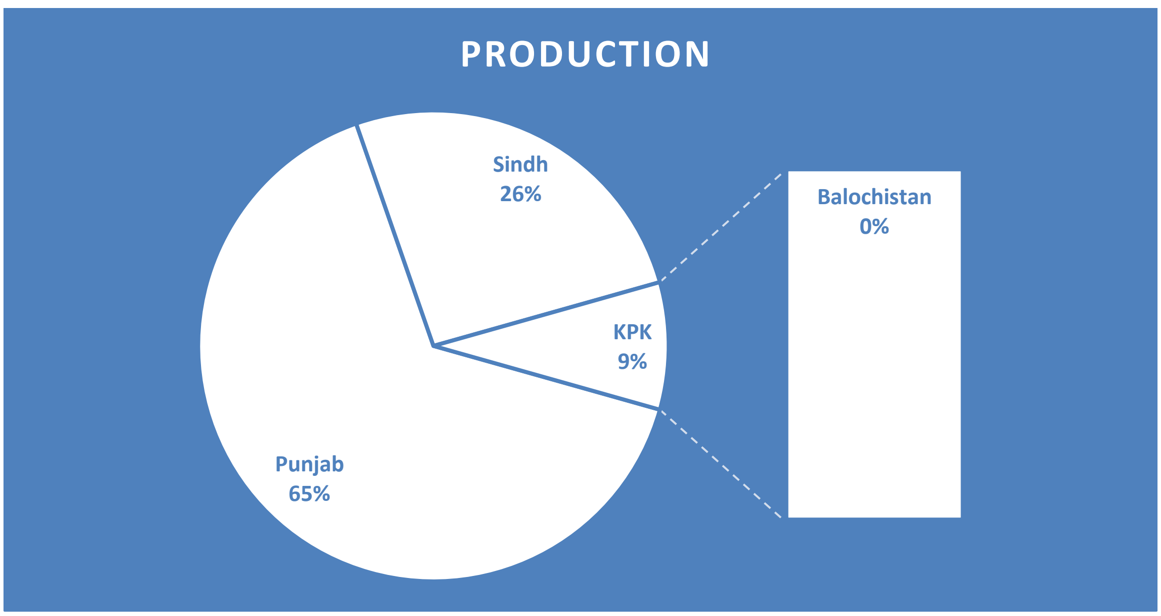

Fig 1. Province wise contribution in production of sugarcane in Pakistan

Sugarcane production is impeded by biotic and abiotic factors. More than 100 diseases of sugarcane are caused by bacteria, fungi, phytoplasma, viruses, and nematodes (Bharti et al. 2012). Among fungal diseases, red rot which is also known as cancer of sugarcane (Khan et al. 2011), is the potential threat to the successful production of sugarcane and is caused by

Colletrichum falcaum. It was firstly reported in Java and then spread in all the sugarcane growing areas of the world (Bharti et al. 2012). Major sugarcane growing countries which are affecting by red rot are shown in the Fig 2.

Fig 2. Distribution map of of sugarcane in the world

Epidemiology and mechanism of disease expression

Colletrichum falcaum, which is causal agent of red rot, is primarily disseminated through diseased setts and soil. Whereas, secondary spread, is through rain splashing, irrigation water, midrib lesion, dew brushing and wind (Hassan et al. 2010) but spread of inoculum depends on the time of the year and conditions of environment. Pathogenic fungus infects stalks through leaf scars, nodes, growth rings, buds and root primodia. It enters the nodes of a sugarcane plant through the inner epidermis of the lower part of the leaf sheath and produces appressoria on the rind and leaves under unfavorable environmental conditions. Later in season, pathogen causes infection when placed on healthy leaves of the sugarcane plants. Latent fungal structures such as dense-walled hyphae, appressoria, chlamydospores, and setae play a vital role in the dispersal of disease during soil transmission (Duttamajumder 2008; Viswanathan et al. 2011) but disease appearance depends on environmental conditions and the type of the infection (Satyavir 2003). *C. falcaum* produces a phytotoxic metabolite which is known as anthroquinone. This toxic metabolite of the pathogen is host-specific and has a significant role in symptoms expression of the disease (Malathi et al. 2002). The sexual stage of fungus (*Glomerella* *tucumanensis)* is responsible for the survival of the fungus on decaying leaves and the formation of new virulent pathological races that are responsible for the frequent outbreaks of the red rot.

C. falcaum requires 25-30°C temperature and rainy season for the development of red rot of sugarcane. Typical symptoms of this fungal disease are observed during monsoon and post-monsoon period (Satyavir 2003). Fungus produces conidia which spread through wind and infect the upper parts of the plant(Satyavir 2003) which later on spread on each part of the plant (leaf, shoot & stalk) after the rainy season (Duttamajumder 2008). The spindle leaves of the plant start drying from top to margin, and the stalk of the plant becomes hollow and discolored. Lesions with minute red spots occur in both directions of the upper surface of the lamina and leaves may break and hang down (Duttamajumder 2008). At the advanced stage of red rot, stalk of the plant (which is the most susceptible part) becomes hollow and covered with white mycelial growth of the fungus and produces alcoholic order in the juice of the diseased cane. Mycelium of the fungus spread cell to cell, leading to white gummy spots. Sucrose of the diseased plant is hydrolyzed by an enzyme "invertase" and converts sucrose into fructose/glucose which results in, the increase in molasses. Reddening of the internal tissues of sugarcane is the typical symptom of the red rot of sugarcane disease that develop only at later stages (Nithya et al. 2012). The pictorial description of disease cycle of *C. falcatum* is shown in Fig 3.

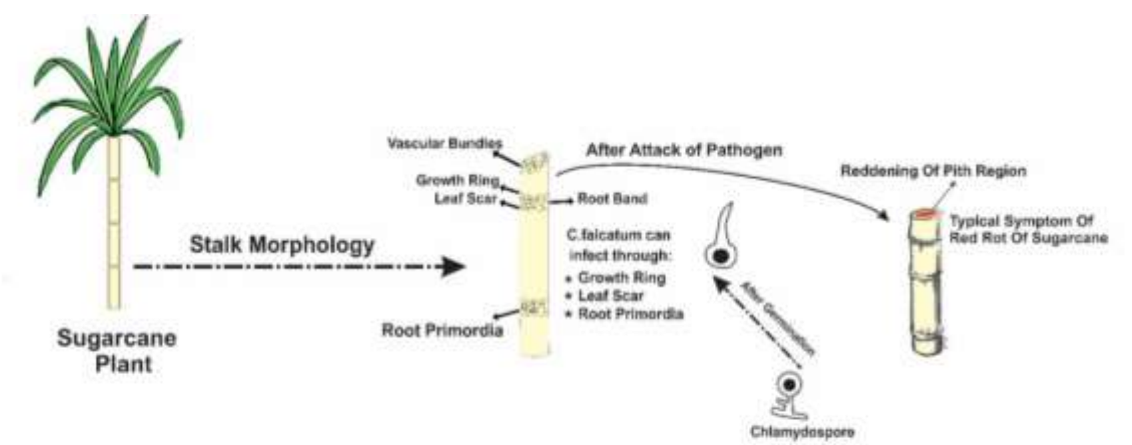

Fig 3. Disease cycle of red rot of sugarcane under field conditions

Management tactics towards *Colletotrichum falcatum* causing red rot of sugarcane

i. Cultural practices towards red rot of sugarcane

Healthy cultural practices should be adopted while planting the sugarcane. Use of disease-free seed, field sanitation, crop rotation and efficient drainage have been suggested to minimize inoculum in the field. Infected seed material is source of primary inoculum. That's why disease-free seed should be used (Satyavir 2003). In this context, sanitary measures must be adopted in the field and crop debris, trash and stubble should be burnt prior to planting (Sharma and Tamta 2015). Ratooning should be avoided in case of heavily infected plant crops. Crop rotation should be adopted by growing some other crop for 2-3 years to reduce the soil borne inoculum Flow of irrigation water from infected to healthy plants should be avoided. Long setts should be used for planting and 3 or 4 budded sets are very suitable seed setts (Anwar et al. 2010).

ii. Chemotheuroptic approach to manage red rot of sugarcane

The use of resistant varieties is the most effective method to control red rot (Viswanathan et al. 2011) because incidence of plant diseases can be reduced through the use of healthy seed, crop rotation, field sanitation and destruction of the diseased planting material, crop debris, and stubbles (Satyavir 2003). Long sets should be given preference for planting to manage soil-borne inoculum of *C. falcatum* (Anwar et al. 2010). No doubt, use of resistant source and cultural practices to manage red rot is the most economical, easy and ecofriendly but if the diseases appeared suddenly in epidemic form then use of fungicide is the only option for farmers to protect their crops from harms of the red rot.

Application of chemicals, such as Benomyl and Carbendazim applied on infected sets reduce incidence of red rot of sugarcane disease. Similarly, Carbendazim and Thiophanate methyl have been tested to control *C. falcatum*. It has been observed that 0.25 % Thiophanate methyl reduced the incidence of red rot up to 75% (Malathi et al. 2002; Subhani et al. 2008). Chemicals like Foliar, Ridomil & Benomyl also expressed significant reduction in diseases development while Vitavax and Bavistin inhibited, 91 % and 98% growth of fungus respectively under *invitro* conditions (Bharti et al. 2014). Similarly, Difenoconazole 11.4% SC (@ 1.00 ml/L and Azoxystrobin 18.2 % can be used to control *C. falcatum* (Shailbala et al. 2019). Seven fungicides @ 10 ppm, 15 ppm, 20 ppm, and 25 ppm were tested. All fungicides exhibited significant reduction in mycelial growth of *C. falcatum* under lab. conditions (Ghazanfar et al. 2017). A summary of different fungicidal effect at different concentrations and active ingredients along with their mode of action is represented in the Table 1 which were evaluated by various scientists in different localities of the world towards *C. falcatum* causing red rot of sugarcane. While mode of action of action of some effective chemicals towards *Colletotrichum falcatum* causing red rot of sugarcane is shown in Fig 4.

Table 1. **Summary chemicals along with active ingredients and mode of action evaluated by various scientists towards *Colletotrichum falcatum* causing red rot of sugarcane**

Trade Name	Active Chemical	Mode of action	Concentration	Reference
Score	Difenoconazole	This fungicide stops the development of pathogenic fungi by interfering with the biosynthesis of sterols in the cell membrane of fungi.	11.4% SC (@ 1.00 ml/1)	(Shailbala et al. 2019)
Ridomil Gold	Mancozeb	This fungicide inhibits the germination of pathogenic fungal spores.	3%	(Abbas et al. 2016)
Bavistin 50DF	Bavistin	This fungicide (Bavistin) can inhibit the germination of pathogenic fungal spores and it disturbs the metabolism of fungus by interfering with the cell membrane.	50 ppm	(Bharti et al. 2014)
Topsin M	Thiophanate methyl	This fungicide (Thiophanate methyl) binds to tubulin and blocks mitosis.	0.25%	(Subhani et al. 2008)
Blue Shield DF	Copper oxychloride	This fungicide (Copper oxychloride) inhibits the germination of pathogenic fungal spores.	1%	(Abbas et al. 2016)
Amistar Top	Azoxystrobin	This fungicide prevents respiration of fungi because of disruption of ETC (Electron Transport Chain).	23%	(Shailbala et al. 2019)
CabrioTop 600WDG	Metriam+ Pyraclostrobin	It ensures a longer protection duration by penetrating the leaf tissues and deposition in the waxy layer.	25ppm	(Ghazanfar et al. 2017)
Dhanuka Vitavax power	Vitavax	It protects the emerging seedlings and seeds from fungal attacks.	100ppm	(Singh et al. 2014)
Nimrod 25 EC	Bupirimate	It penetrates through the leaf to kill the existing infection caused by fungi.	50 µg mL-1	(Subhani et al. 2008)

Tilet 250 EC	Propiconazole	It stops the development of fungus and interferes in the biosynthesis of sterols in cell membranes..	20 and 50 µg mL-1.	(Subhani et al. 2008)
Aretan, Agall ol	Methoxy ethyl mercuric chloride	It acts by reacting with the sulfhydryl group in fungal pathogens and inhibits the growth	0.25%	(Satyavir 2003)
Nativo®WG 75	Trifloxystrobin+Tebuco nazole	It acts as a respiration inhibitor. It blocks the electron transfer at the mitochondrial membrane of the fungus.	25ppm	(Ghazanf ar et al. 2017)
Topas 100 EC	Penconazole	It suppresses the development of fungus by interfering with the biosynthesis of sterols in cell membranes.	20 µg mL-1	(Subhani et al. 2008)
Thiram	dimethyldithiocarbamat e (dimethyl dithiocarbamate)	It suppresses the sulfhydryl enzyme and metal-dependent systems in fungus.	1000 ppm	(Kumar et al. 2015)
Fundazol 50 WP	Benomyl	It inhibits the mitosis in fungus.	1.5 g / liter of water	(Khan et al. 2009)
Agrimycin-100	Oxytetracycline hydrochloride	It inhibits cell growth by inhibiting the translation process.	1500 ppm	(Valarma thi et al. 2020)
Rubigan 12EC	Fenarimol	It works by stopping the biosynthesis of significant steroid molecules of fungus.	50 µg /mL	(Subhani et al. 2008)

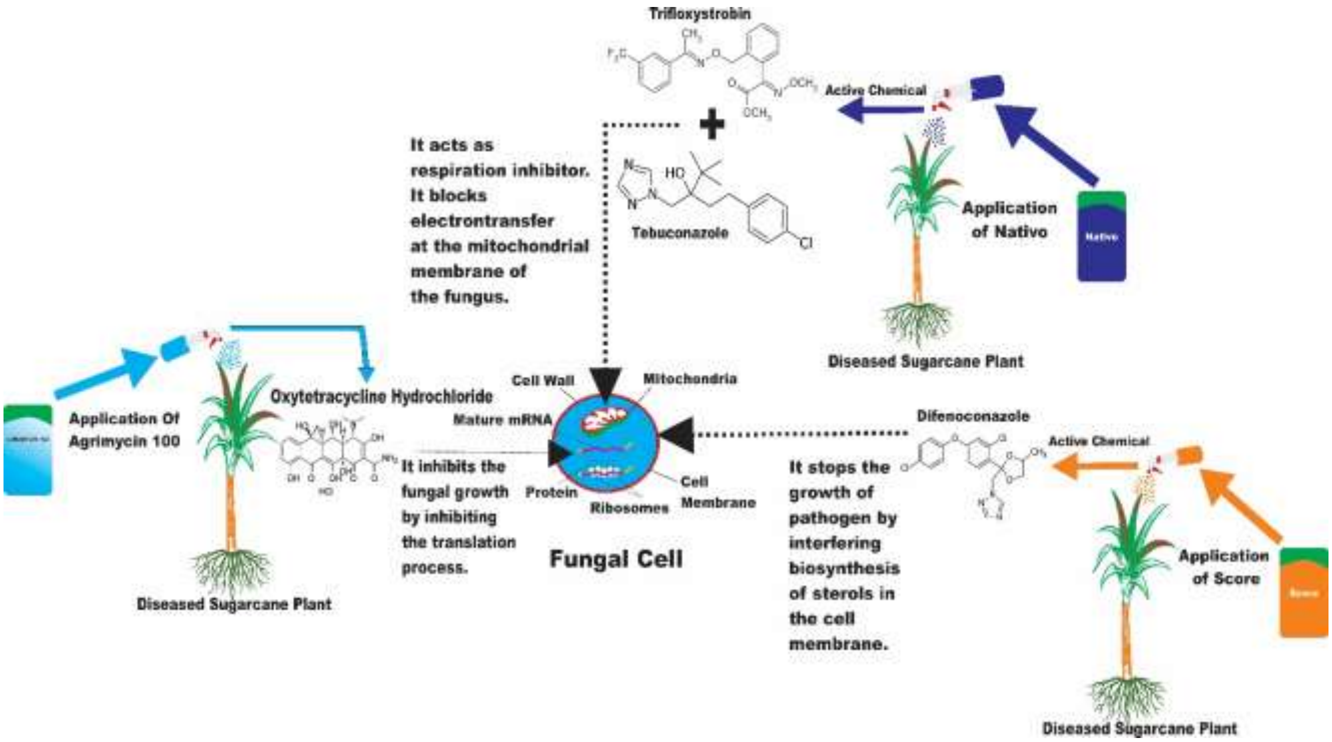

Fig 4. Mode of action of some effective chemicals towards *Colletotrichum falcatum* causing red rot of sugarcane

iii. Phytochemicals as a management tool towards red rot of sugarcane

For management of plant diseases, farmers mainly depended on fungicides but their continuous use developed resistance in pathogens and causes environmental pollution. This thing motivated scientists, researchers and farmers to explore some other possibilities to manage plant diseases. In current scenario, use of phytochemicals towards plant diseases is appropriate method as plant extracts contains multiple defensive compounds with different mode of action towards pathogens. These phytochemicals not only contain antimicrobial compounds but also contain a number of chemical compounds which not only boosts up resistance mechanism of the host plants towards pathogen but also improves the yield of the host crop (Shupping and Eloff 2017).

The use of phytoextracts to control plant diseases is being given significance. It is very important to search out different phytoextracts to evaluate their potential against plant diseases including pests in the field of agriculture that are eco-friendly and less harmful to the health of human beings (Nasir et al. 2014). It has been found that the crude extracts of approximately twenty-five Indian ornamental plants have the potential to control the germination of pathogenic fungus. Research reveals that ethanol extracted from *sargassum myricocystu* (brown alga) have shown significant result to manage the mycelial growth of pathogenic fungus (Ambika et al.

2015). It has been reported that spore germination including mycelial growth of pathogen inhibited 100% through phytoextracts in the lab (Rajkumar et al. 2013). Various medicinal plants such as (Vitex negundo, Piper betel & Aloe vera) have been tested to fight against *C. falcatum* (Prabakaran and Prince 2011). It has been observed that fruit extract of *M. citrifolia* can reduce the growth of fungus *C. falcatum* that is an etiological agent of red rot of sugarcane disease (Jainkittivong et al. 2009). Extracts of five plants such as *Gliricidia sepium, Allium sativum, Saussurea lappa, Azadirachta indica* and *Curcuma Longa* have been tested in 3 different concentrations 5.0%, 10.0%, and 15.0% against pathogenic fungus *Colletotrichum falcatum* for evaluation of their antifungal activity under invitro conditions (Tariq et al. 2017). All the phytoextracts (*Gliricidia sepium, Allium sativum, Saussurea lappa, Azadirachta indica* and *Curcuma Longa*) inhibited the fungal mycelial growth to some extent. Inhibition of mycelial growth of the pathogenic fungus was detected at 15% of *Gliricidia sepium*, *Curcuma longa*, *Saussurea lappa* and *Azadirachta indica* up to 59.23%, 79.23%, 83.64%, and 93.25% respectively (Tariq et al. 2017). The efficacy of the three types of leaf extracts such as mixed leaf extracts, neem leaf extracts including bitter leaf extracts has been tested to evaluate their antifungal activity against pathogenic fungus (Olahan et al. 2020) also shown in Table 2. For better understanding mechanism of microbial growth inhibition by garlic extract is shown in Fig 5.

Table 2: **Summary about efficacy of phytochemicals used against red rot of sugarcane**

Phytoextracts	**Active ingredient**	**Mode of action**	**Reference**
Aloe vera extract	Phenolics	Phenolics act to denature and coagulate proteins	(Prabakaran and Prince 2011)
Fruit extract of M.citrifolia	Hexanoic acid	It alters fungal membrane permeability.	(Jainkittivong et al. 2009)
Neem Extract	Quercetin and β-sitosterol	It has the potential to suppress mycelial growth.	(Tariq et al. 2017)
Bitter leaf extracts	Phenolic acids	It acts to denature and coagulate proteins.	(Olahan et al. 2020)
Garlic extract	Allicin	It inhibits the mycelial growth of fungus.	(Tariq et al. 2017)

Argemone Mexicana	Alkaloids	Alkaloids act as defense compounds against fungus.	(Adebola et al. 2016)
Curcuma longa	Curcuminoids	It disrupts the fungal cell membrane.	(Tariq et al. 2017)
Carica papaya	Terpenes	It inhibits the germination of fungal spores by shortening germ tubes and degrading the tips of hyphae.	(You et al. 2011)
Z. spina-christi	Saponins	It alters the morphology of the fungal cell wall and increases the amount of chitin that kills fungus.	(Jiya 2021)
Saussurea lappa	Costunolide	It inhibits mycelial radial growth	(Tariq et al. 2017)
Corchurus olitorius	Polyphenols	Polyphenols are related to defense responses in plants; through different mechanisms such as preformed inhibitors or response-synthetized compounds against fungus.	(Guzzetti et al. 2021)
Dhatura metel	Amides	It effects on the morphology of the plasma membrane and the cell wall of the fungus.	(Patel et al. 2015)
Cymbopogan citratus	Citronellol	It inhibits biofilm and secrets phospholipases and extracellular proteinases	(Adebola et al. 2016)
Brassica oleracea	Glucosinolates	It plays a vital role in plant defense.	(Patel et al. 2015)
Gliricidia sepium	Sesquiterpene hydrocarbons	It disrupts the cell wall of fungi.	(Tariq et al. 2017)
Alpinia galanga	1'-acetoxychavicol acetate	It inhibits the *Mycelial Growth*	(Patel et al. 2015)
Lawsonia inermis	Ethanol	The mode of action of extracts was determined on the cell wall and enzyme production of fungi.	(Jiya 2021)

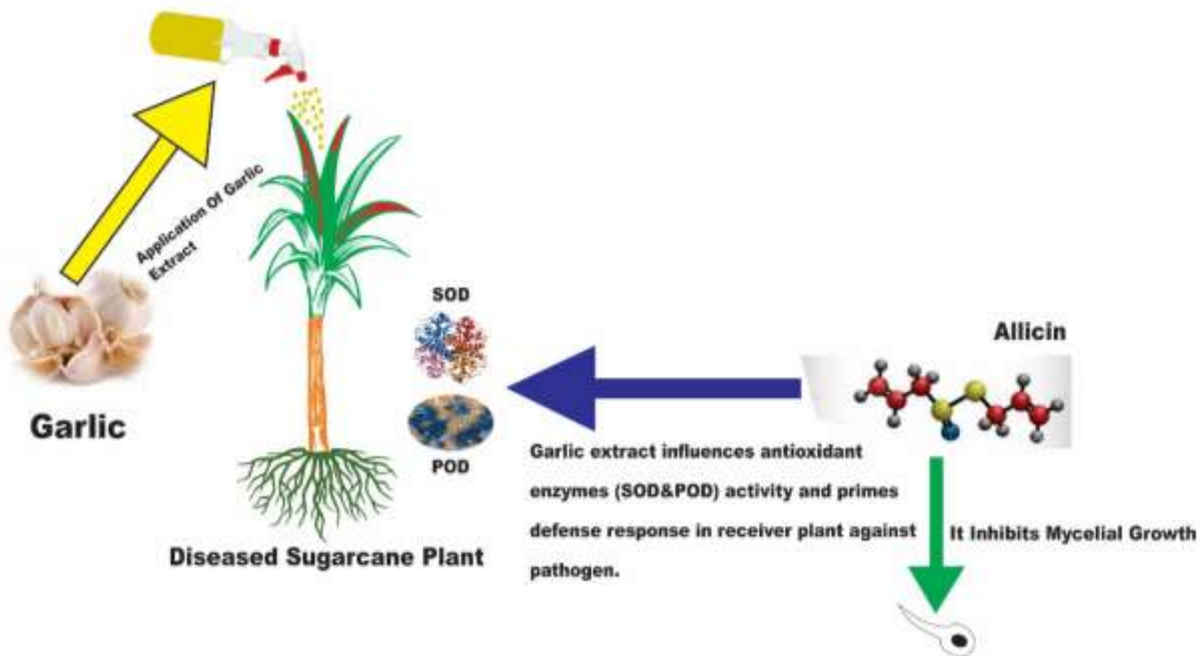

Fig 5: Mode of action of garlic extracts towards *Colletotrichum falcatum* causing red rot of sugarcane

iv. **Eco-friendly Strategy to overcome red rot disease (Bio control)**

Now a days people of the world are health conscious, so they prefer to use those fruits are vegetable which are free from chemical sprays against diseases. No doubt, chemicals expressed rapid reduction in diseases but have health hazardous effects on human beings due to residual effects of chemicals. This this enforces scientist and researches to find out alternative to chemicals and the use of biocontrol agents or their metabolites, is the appropriate way to address pathological issues of the sugarcane as it is reported by Fravel (2005). *Trichoderma viride* and *Trichoderma harzianum* and *Pseudomonas* sp. have potential to control *C. falcatum* due to production of chitinase enzymes with an antifungal potential (Singh et al. 2008). Antagonistic activity of fungal strains such as ARSEF-6647, ARSEF-6648, ARSEF-6646, ARSEF-2417, and ARSEF-6650 of *Beauveria bassiana* have been studied. These antagonistic fungal strains produce chitinolytic enzymes and cause lysis of the mycelium of pathogenic fungus (Sanivada and Challa 2014).

For integrated management of red rot of sugarcane, studies were conducted to evaluate the compatibility of antimicrobial agents with fungicides to control pathogens. The addition of 1ppm thiophanate methyl influenced the antagonistic activity of the Trichoderma strains and it caused suppression of pathogen growth more aggressively. The addition of fungicide at low concentration enhanced antimicrobial activity of Trichoderma spp by weakening the *C. falcatum* (Malathi et al. 2002). It has also been observed that the addition of salicylic acid to the culture of *T. harzianum* enhances its mode of action towards C. *falcatum*. According to previous research, Pseudomonas strains such as VPT4, KKM1, EP1, and CHAO induced systemic resistance against *C. falcatum* in susceptible cultivars of sugarcane (Viswanathan et al. 2011). Soil application of pseudomonas strains such as Pfl, CHAO, EPI, and *Pseudomonas fluorescens* putida strains KKMI , twice in a year suppressed the red rot but also heightens the yield of sugarcane (Senthil et al. 2003) while Singh et al. (2008) noted reduction in incidence of red rot and enhancement in cane yield when *Trichderma viridae* along with carbendzim and MHAT applied in the field. General mode of action of bio-control agents is shown in Fig 6.

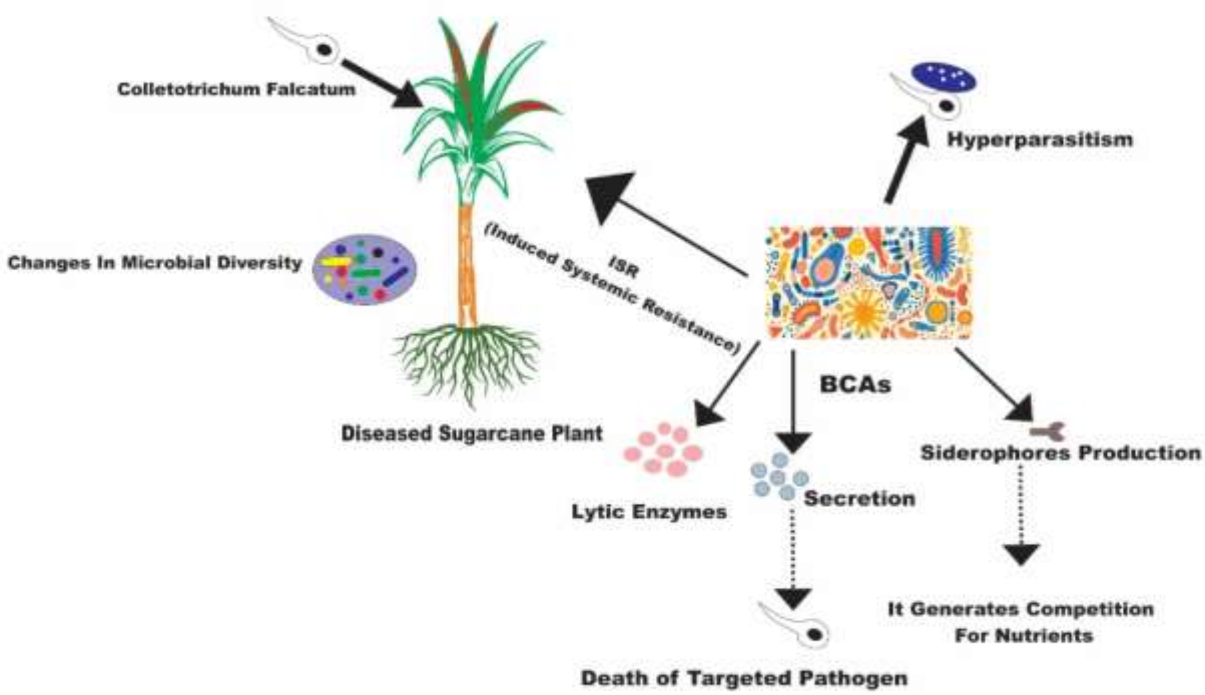

Fig 6. General mode of action of biocontrol agents towards *Colletotrichum falcatum* causing red rot of sugarcane

v. Nanotechnology: A recent tool towards red rot

Commonly, plant disease management relies predominantly on toxic chemicals which are potentially harmful to environment and the humans. Nanotechnology is an emerging tool which opens a wide array of opportunities in the field of agriculture; especially it is being preferred in plant disease management. It can offer advantages to pesticides such as increasing the solubility of poorly water-soluble pesticides, reducing toxicity and improving the shelf-life, all of which could have positive impacts on environment.Of the several nanoparticles that exist, only silver (AgNPs), copper (CuNPs) and zinc nanoparticles (ZnNPs) have received much attention due to their antimicrobial activity. The first nanoparticles to be studied in plant disease management were AgNPs (Park et al. 2016). The efficacy of silver nanoparticles has been evaluated against fungal pathogens in various studies (Mallaiah 2015). Recently, the application of silver nanoparticles (AgNPs) is proceeding because of incredible utilization in different fields. Some pathogens have been reported to express the development of resistance against various antimicrobial agents including pesticides but failed to do so, against silver nanoparticles (Bratovcic 2020). Sugarcane husk, rhizobacteria, and *Fusarium oxysporum* have been used in the synthesis of silver nanoparticles and their antifungal activity has been investigated against *Fusarium moniliforme* and *C. falcatum* under *in vitro* conditions (Mahmood et al. 2021). For sustainable management, biosynthetic silver nanoparticles were prepared from Bacillus spp and were studied for their antifungal activity towards pathogen. It was observed that AgNPs suppressed the mycelia growth along with carbendazim more efficiently (Ajaz et al. 2021).

Previous research showed that ZnO-NPs (zinc oxide nanoparticles) expressed antifungal potential and *Colletotrichum* spp have been exposed to the action of concentrations of 730 ppm, 980 ppm including 1200 ppm of zinc oxide nanoparticles and compared with the effect of the ciproconazole (+ve control) as well as with normally grown fungus (-ve control). The zinc oxide nanoparticles showed an excellent result by inhibiting the fungal growth up to 96% at a concentration of 15 $mmolL^{-1}$ (Mosquera-Sánchez et al. 2020). Antifungal activity of ZnO-NPs and MgO-NPs on *Colletotrichum* strains obtained from tropical fruits have been studied. The zinc oxide nanoparticles including magnesium oxide nanoparticles proved effective by inhibiting 88% fungal growth. The zinc oxide nanoparticles are an efficient economical, and viable antifungal alternative to be used in crop protection systems (Rosa-García et al. 2018). Similarly antifungal potential of Silica- Silver NPs was detected to suppress the growth of fungal pathogens such as *Colletotrichum* (Park et al. 2016). Silver (Ag) exhibits significant antimicrobial activity and research efforts on it as a substitute for chlorine and other toxic nanomaterials has been progressing (Park et al. 2016). Nanoparticles kill the pathogens by cell lysis because these are extra small in size, which make holes in the cell membrane of microbes (Fig 7).

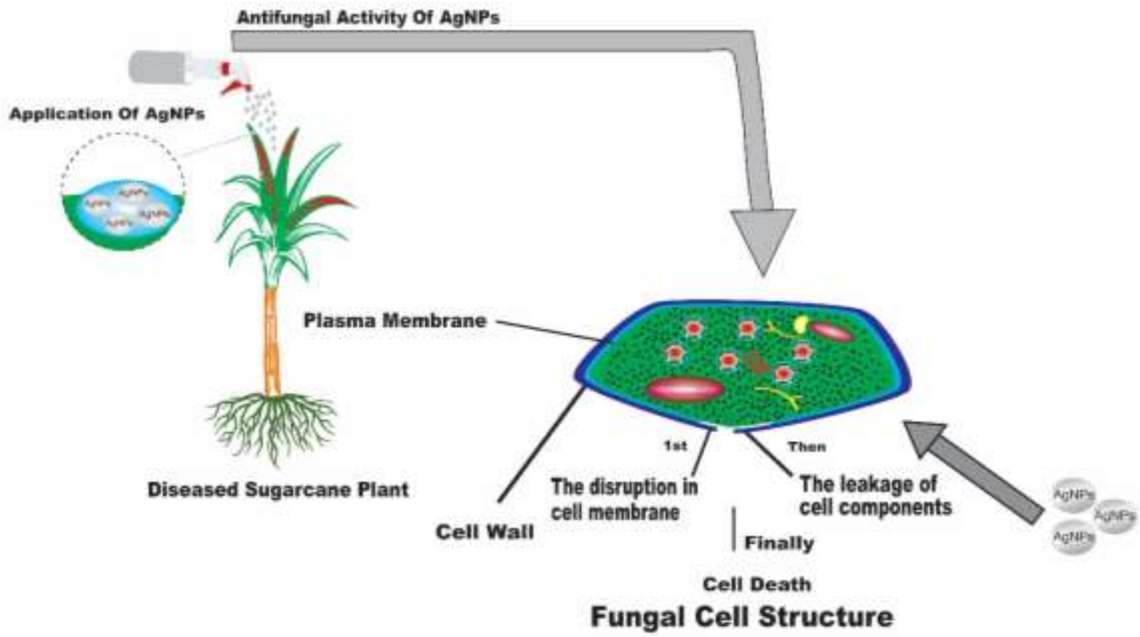

Fig 7. Mode of action of AgNPs towards *Colletotrichum falcatum* causing red rot of sugarcane

Future Direction and suggestions towards red rot of sugarcane

It is the need of the hour to direct the efforts on cutting-edge research areas especially in Pakistan. There is a dearth of information on early detection and strategies to fight against *C. falcatum* because of many overlapping characteristics within the species complex. Work on pathotyping should be done because of the complex and diverse nature of the pathogen. Briefly, an intense genome project on this pathogenic fungus is urgently needed. It has been detected that introduction of new pathogenic strains has been blamed for the withdrawal of varietal resistance to red rot of sugarcane disease. There are pathogenicity variations in *C. falcatum* that's why my opinion is that enhancing the resistance of sugarcane to the pathogen is the foremost economical and effective approach for controlling this disease. Researchers are using the CRISPR/Cas9 technique in several agricultural plant spp. through targeting several genes of interest for improving nutrition, tolerance and resistance against plant pathogens. So it is the need of hour to improve resistance in the sugarcane crop through CRISPR/Cas9 technique towards red rot diseases

A comprehensive study regarding the impact of epidemiological factors and the development of disease forecasting models should be done. Disease forecasting models should be introduced at region-level by keeping in view ecological conditions. For eco-friendly management, it is vital to explore the potential of endophytes towards *C. falcatum* . Various BCAs do not have the potential to thrive in newly introduced habitats that's why, it is also vital to introduce new bio control agents to contest this ravaging pathogen. Verily, bio control strategy is promising but it requires extensive field evaluation to develop bio-formulated products. Fungal and bacterial metabolites have the potential to display antifungal activity against red rot-causing pathogen. The continuous research efforts to evaluate the efficacy of fungal and bacterial metabolites that act as an inhibitor of *C. falcatum* should be an alternative and effective strategy in a broader context. Other investigations need to be carried out to determine the suitable conditions for the application of these metabolites to get the higher antifungal effect. Very limited work has been done on the Nano technological approach for disease management. Nanotechnology is a rapidly evolving discipline for plant disease management and this Nano technological approach may prove a fruitful tool to combat this phytopathological challenge. It is need of the hour to use loop mediated method for early detection of the pathogen for accurate and in time management of red rod of sugarcane to reduce economic losses of the farmers.

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