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Sustainable Management Approaches of *Colletotrichum falcatum* in Pakistan

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ABSTRACT

Sugarcane (*Saccharum officinarum*) is a vital member of family Poaceae. It is cultivated worldwide to run the sugar industry and for its nutritional properties. Biotic and abiotic factors have posed obstruction in the sustainable production of sugarcane. Among biotic factors, red rot caused by *Colletotrichum falcatum* has been proved to be the most ravaging biotic stress of the sugarcane crop. The sugarcane industry currently lacks a sustainable method for controlling red rot expansion in the field, resulting in the abandonment of many sustainable sugarcane varieties. Integrated Disease Management (IDM) is in some way a good technique to prevent red rot in the fields. Combination of different management techniques like use of resistant variety with hot water treatment, use of chemical and biological control and use of nano-pesticides can at some point prove to be a good technique for red rot prevention in the fields. Objective of current research is to highlight early detection of disease in the fields, assess their mode of transmission of *C. falcatum* in the field and to highlight different management strategies for the prevention of the disease. The implementation of our proposition could have a significant impact on the mitigation of *C. falcatum* infection in the sugarcane industry, thereby enabling sustainable sugarcane cultivation and guaranteeing the long-term viability of sugarcane industry in tropical and subtropical regions.

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INTRODUCTION

Sugarcane (*Saccharum officinarum*), a member of the Poaceae family, is an important perennial C_4 crop that is widely produced around the world . Its prime origin is Austronesia. Later, it was introduced to Polynesia, Island Melanesia, and Madagascar in prehistoric times via Austronesian sailors (Punda *et al*., 2009). The statistical report of Food and Agriculture Organization stated that worldwide sugarcane is cultivated on an area of 26 million hectares with a production of 1800 million tons. Average sugarcane production worldwide is 60 tons/hectare. Major sugarcane producing countries are Brazil, India, China, Pakistan, and Thailand. respectively. Share of America and Asia is 92% of the sugarcane production across the globe, while in Pakistan, it is cultivated on an area of 1.260 million hectares with an annual production of 88.654 million tons. The Punjab Province accounts for 60-65 %, Sindh 25-30 %, Khyber Pakhtunkhwa 10 %, and Baluchistan less than 1 % of the area under sugarcane cultivation. Average production of sugarcane in Pakistan is 46 tons/hectare. Its production accounts for 3.4% in agriculture's value addition and 0.7% in GDP. Sugarcane being 2nd largest agroindustry in the country, is a source of livelihood for millions of people (Muhammad *et al*., 2021).

It is a good source of minerals and vitamins like Ca (2%), Fe (9%), Mg (4%), P (3%), K (3%), Zn (1%), vitamins B_6 (31%), B_9 (11%) and C (8%) also Carbohydrates, fats, and proteins (Cantwell-Jones *et al*., 2022). However, it also possesses several medicinal properties that have been used for centuries in traditional medicine (Ahmad *et al*., 2023). Sugarcane juice is rich in antioxidants, minerals, and soluble fiber, making it a potential natural remedy for various ailments (Wang *et al*., 2018). Sugarcane juice can boost the immune system, prevent tooth decay, relieve constipation, and treat jaundice by providing body with vitamin C and hydration. Moreover, it can provide natural glucose to the body, reduce inflammation, prevent cancer, and lower cholesterol levels (Muhammad *et al*., 2021). By-products of sugarcane include sucrose, jaggery, syrups, cellulose, green leaves, bagasse, molasses, alcohol, press mud and spent wash (Dotaniya *et al*., 2016).

Sugar is a ubiquitous component of our daily lives, however, there is an increase fascination with sugarcane as a promising economic crop to produce bioenergy (ethanol) (Gianotto *et al*., 2011). Sugar production in Pakistan in 2023-24 is forecasted at 7.05 million tons, 3% above the 2022-23 estimate. This growth can be attributed to the anticipated recovery in cane area harvested following the flood induced damage during the previous year's crop. Concurrently, sugar consumption for 2023-24 is expected to rise 6.3 million tons, representing a 3 % increase from 2022-23 levels, primarily driven by population growth. However, according to United States Department of Agriculture, in an effort to prevent domestic shortage and price surges, sugar export for 2023-24 are anticipated to reach 800,000 tons, slightly lower than the previous year, as the government implement measures to regulate and limit export volumes.

Sugarcane productivity has decreased due to biotic (bacteria, fungi, nematodes, virus) and abiotic (heat, drought, cold, frost, floods, salts) factors. There are approximately 240 sugarcane diseases which affect sugarcane lifecycle (Hossain *et al*., 2020). About 100 fungi, 10 bacteria, 50 nematodes and 10 viruses have been identified as pathogens of sugarcane worldwide (Sharma and Tamta, 2015). Among biotic factors, red rot, wilt, whip smut, sett rot, sugarcane streak mosaic, sugarcane mosaic virus, yellow leaf syndrome are the significant diseases of sugarcane (Thilagavathi, 2020). Among all these diseases, red rot of sugarcane caused by *Colletotrichum falcatum* is the most devastating disease. It causes a decrease in yield by 5-50%, cane weight by 29%, sugar recovery by 31%, sucrose content by 75% and sugarcane juice by 90 (Hossain *et al*., 2020). Overall disease incidence of red rot of sugarcane in Pakistan was recorded to 45.60 %. Recently, it has caused damage at extreme level and termed as the most pernicious and important danger in cultivation of sugarcane in Pakistan (Ahmad *et al*., 2023).

MATERIALS AND METHODS

Pathogen

Morphological characteristics

Colletotrichum falcatum is a fungus of the phylum Ascomycota, class Sordariomycetes, order Glomerellales, family Glomerellaceae and genus Colletotrichum. It is a saprophytic soil-born fungus. It occurs in both anamorphic and teleomorphic forms, but anamorphic stage is the most important, as it infects standing canes (Viswanathan *et al*., 2019). Hyaline, linear, or club-shaped conidiophores serve as its asexual fruiting bodies, from which it produces solitary, colorless conidia with extremely thin walls and a single cell (Ali *et al*., 2021). *Colletotrichum falcatum* is a fungus with a mycelium that is immersed, branched, septate, and hyaline to dark brown in color. Acervuli are subcuticular, epidermal, or sub-epidermal structures that can occur as distinct units or merge into a continuous mass, composed of cells exhibiting variable pigmentation, ranging from hyaline to dark brown, with thin or thick cell walls, and irregularly dehisce. Sclerotia may be present in culture, appearing dark brown to black, often coalescent, and occasionally bearing setose appendages. Setae within acervuli or sclerotia are brown, smooth, septate, and acutely pointed at the apex. Conidiophores, originating from the upper stromal cells, from a dense, even stand and can be simple or basally branched, aseptate or septate, and exhibit variable hyaline to brown pigmentation. Conidiogenous cells are hyaline, aseptate, with a straight to falcate shape, smooth, thin walls, and occasionally feature an apex drawn into a cellular appendage. Appressoria, designed for surface attachment, are brown, either entire or crenate along their margins, and can remain simple or undergo repeated germination to yield complex columns or interconnected appressoria (Sharma *et al*., 2017).

Isolates

Numerous fungal isolates show significant variations. Within pathotypes, diverse levels of virulence have been observed, indicating that *C. falcatum* adopts to different host cultivars (Bharti *et al*., 2012). Vishwananthan *et al*., 2019 reported that these isolates exhibit virulence in susceptible varieties but not in resistant and moderately susceptible varieties. The frequency of virulence among isolates ranges from 21.3% to 40% on moderately susceptible varieties, but they are much higher, ranging from 62.9% to 97.9%, on susceptible varieties. These results highlight the considerable diversity in host infectivity among *C. falcatum* isolates. Gaining a comprehensive grasp of the diversity of the pathogens and the methods by which they infect is essential for the formulation of successful disease control measures and the establishment of well-informed agricultural practices. By identifying the factors that contribute to varying virulence levels, it becomes possible to devise targeted strategies to mitigate red rot disease and protect susceptible crops while preserving resistant or less susceptible cultivars (Cooper *et al*., 2011).

Epidemiology

Colletotrichum falcatum development is affected by various parameters of environment like temperature, relative humidity (%) and soil pH (Ghodake *et al*., 2019). These factors are known to affect the growth stage, host susceptibility, vigor, survival, rate of multiplication and rate of spore penetration and germination (Freitas *et al.,* 2019). Mean temperature range of 27.5-32.5 °C and relative humidity of approximately 90% are conducive for disease development (Duttamajumder, 2008).

Transmission

Initial dissemination of the *C. falcatum* pathogen occurs via the soil and diseased setts, while secondary dissemination occurs via aerosol and precipitation (Hassan *et al*., 2012). Monsoon season is the most appropriate season for disease development. Fungus is transmitted in a secondary manner during the monsoon through rain (Cioffi *et al*., 2004). In the summer environmental conditions prevalent in fungi promote infection and become significant threat to cane cultivation. *C. falcatum* can enter the plant via natural openings or injuries caused by improper handling of blades during planting or by sugarcane borer (*Diatrea saccharalis*) leading to the borer-rot disease complex (Costa *et al*., 2021). *C. falcatum* infects and damages sugarcane plants in a complex mechanism (Amna *et al*., 2021). *C. falcatum* produces enzymes (cellulases, pectinases, ligninases, proteases and lipases) that can break down the cell walls of the plant, allowing it to penetrate the internal tissues. Once inside the plant, the fungus produces toxins that can damage the plant cells and interfere with the normal functioning of the plant. These toxins can also trigger an immune response in the plant, which can lead to the death of infected cells and the formation of lesions on the stem (Katiyar *et al*., 2017). *C. falcatum* can also alter the metabolism of the sugarcane plant, by producing enzymes (hydrolase) that break down sucrose into glucose and fructose. This can result in reduced sugar content in the infected sugarcane stalks (Ponmurugan *et al*., 2016).

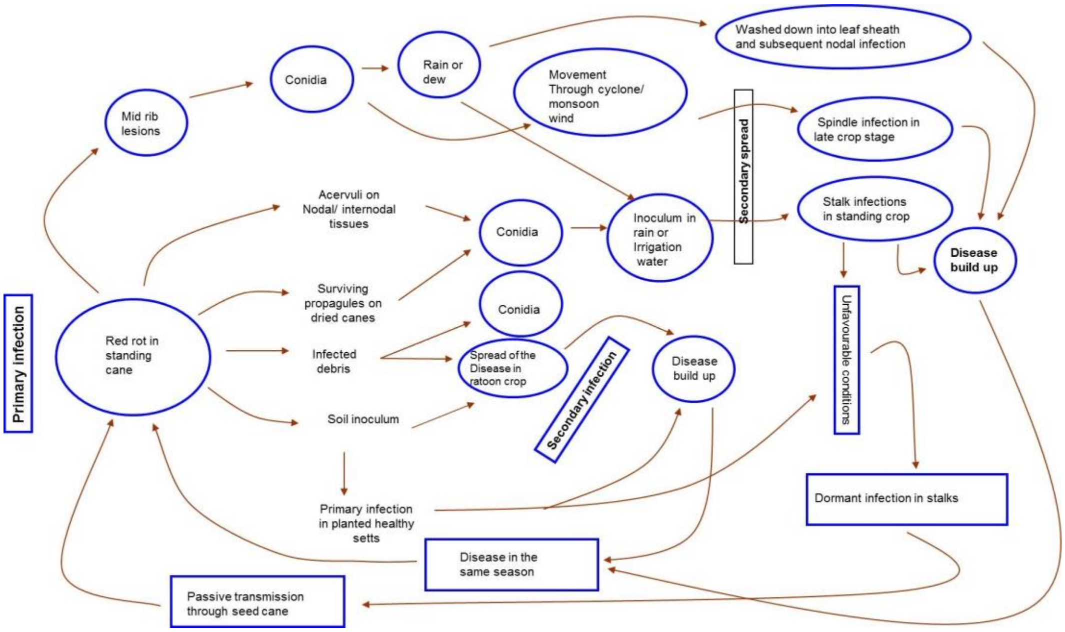

Figure 1. Life cycle of *Colletotrichum falcatum*.

Symptoms

Colletotrichum falcatum is known as a stalk and seed disease because it primarily infects different parts of the sugarcane plant, with a particular preference for the stalks, stubble rhizomes, and leaf midribs. Although, it can extend its invasion to include leaf blades and leaf sheath tissues, it is not regarded as a major concern for the health of sugarcane roots. The typical manifestation of the disease involves the appearance of symptoms on the midrib of leaves and stalks. Initially, the disease exhibits its effects by causing the death of young and emerging shoots, particularly during the months of March and May. However, it is worth noting that these symptoms may not be easily identifiable at first glance (Duttamajumder, 2008).

Stalk symptoms

In the early stages, symptoms may not be evident in the field, making it challenging to detect. As the disease progresses, signs of infection become more apparent. Infected plants exhibit symptoms such as yellowing, withering and desiccation of their upper foliage. The margins of the 3rd and 4th leaf from the crown may dry up. To diagnose red rot in sugarcane, one can accomplish this by splitting the standing cane stalk, uncovering longitudinal reddening within the typically white or yellowish-white inner tissues. These reddish streaks are occasionally interrupted by white patches along the stalk. Moreover, externally, brown or reddish-brown stripes become visible at the nodal regions, and a distinct sour, alcoholic odor emanates from the affected tissue. As the disease advances, minuscule acervuli emerge on the outer surface of the upper internodes, followed by the development of a cottony gray fungal mass in the pith region, which yields a profusion of spores. Rotting occurs during the August-September growth phase. Initially, the disease is undetectable in the field, but as internal tissues suffer extensive damage and decay, some discoloration of the rind becomes visible. The consequences can range from the death of a few plants or clumps to the complete failure of the crop. Lighter-colored sugarcane genotypes may have more noticeable discoloration (Saksena *et al.*, 2013).

Figure 2. Internal symptoms of red rot in a sugarcane stalk.

Leaf symptoms

The lesions discovered on leaf midribs begin as initial dark reddish regions that quickly elongate and sometimes extend along the entire length of the inner midrib. In their early stages, these lesions display a vivid red hue with darker edges. As they mature, the central portions transition to a straw-like color. Upon the commencement of fungal fructification, the lesions become adorned with black powdery accumulations of conidia and acervuli, surrounded by margin of dark reddish-brown tint. Typically, a lesion stemming from a single point of infection remains continuous along the midrib. However, there are instances where it fragments into a sequence of red blotches interspersed with seemingly unaffected tissue. These lesions measure approximately 2 to 3 mm in length and around 0.5 mm in width. Additionally, occasional minuscule red spots can be discerned on the upper surface of the midrib (Sharma *et al.*, 2017).

Figure 3. Symptoms of red rot in the midrib of sugarcane leaf.

Management of red rot disease

The management of red rot disease in sugarcane necessitates the implementation of a comprehensive approach that integrates both preventive and curative measures. This disease, induced by the fungal pathogen *C. falcatum*, poses a substantial menace to sugarcane cultivation. Various strategies have been developed with the specific objective of red rot control in sugarcane, and these methods aim to mitigate the occurrence of red rot following replanting, ultimately enhancing the overall productivity of sugarcane plants. However, management strategies to reduce red rot incidence have not been able to provide satisfactory results. While numerous approaches have been proposed to combat *C. falcatum*, such as the application of systemic fungicides, it is important to note that individual methods often prove ineffective. This inefficacy arises from the presence of fibrous nodes, the impermeable nature of rinds, and the water and sugar content within sugarcane setts, which collectively hinder the absorption of fungicides to the necessary concentration levels required for eradicating the pathogen at the site of infection (Viswananthan and Rao, 2011). Integrated Disease Management (IDM) stands out as a highly effective approach in mitigating the impact of red rot in sugarcane. It serves to significantly reduce the incidence of red rot, enhance growth parameters, and improve the performance attributes of sugarcane compared to non-IDM methods. IDM encompasses the integration of a comprehensive set of management practices, as outlined below.

Agronomic and cultural practices

In the context of mitigating red rot incidence, the strategic practice of crop rotation emerges as a pivotal intervention. The persistence of monotypic crop cultivation exacerbates the accumulation of pathogenic inoculum, there by fostering a conducive environment for disease development. To address this concern, the adaptation of crop rotation cycle, spanning intervals of two to three years, is recommended, while simultaneously discouraging ratooning practices. Furthermore, effective disease management necessitates meticulous field hygienic protocols. This encompasses the expeditious collection and incineration of withered and desiccated diseased leaves within the field. In the realm of sugarcane cultivation, meticulous field leveling, and the rigorous maintenance of hygienic cultivation conditions emerge as indispensable measures of disease prevention. These practices collectively contribute to a comprehensive scientific approach aimed at mitigating red rot in sugarcane crops (Sharma *et al*., 2017).

In the context of managing red rot disease, which is predominantly seed/setts borne, it is imperative to adhere rigorously to disease free nursery practices, emphasizing the vital role of authorized enforcement in nursery programs. Ensuring the provision of setts devoid of disease and pests, along with the careful management of varietal mixtures, is paramount. Notably, the most effective strategy of pathogen control involves the utilization of disease-free setts. Geographical origins of pathogen isolates, as noted by (Sandeep and chahal, 2011), do not correlate with their molecular and pathological heterogeneity. Hence, disease-free planting materials are crucial for commercial cultivation.

Field sanitation, including the removal and burial of crop residues, is vital. Maintaining leveled sugarcane fields and up holdings agricultural hygiene are essential. Regular field inspections and removal of diseased plants can reduce red rot incidence. Given the disease's connection to soil nutrient imbalances, precise fertilizer management plays a pivotal role in disease prevention and control (Gupta *et al*., 2018).

Physical treatment

The primary source of pathogen inoculation in red rot disease within sugarcane fields is infected planting materials (Tiwari *et al*., 2017). Scientific research has demonstrated the effectiveness of hear therapy in suppressing sett-borne red rot infections. Talukdar and Alam in 2000 reported complete eradication of sett-borne red rot infections through moist hot air treatment at 54°C for 3 hours with a relative humidity of 95%. Combining heat therapy with chemical treatment in hot water has also shown good results (Thangamani and Thiruvengadam, 2013). Notably, Talukdar *et al*., 2010 findings indicate that subjecting sugarcane to a moist hot air treatment at 54°C for a duration of 2 hours yielded superior results in reducing the incidence of red rot compared to a hot water treatment at 50°C for the same duration. Furthermore, the use of aerated steam at 52°C and the approach involving sett immersion in cold running water for a continuous period of 48 hours, followed by subsequent hot water treatment at 50°C lasting 150-180 minutes, demonstrated efficacy in the complete elimination of the red rot pathogen (Stoll *et al*., 2008). Other recommended practices for red rot management include waste burning, maintaining soil moisture, and timely crop harvesting (Hossain *et al*., 2020). These physical treatments offer eco-friendly, cost-effective solutions, although they are time-intensive in practice.

Chemical control

In the realm of red rot disease management, the utility of foliar fungicides has been notably limited; however, substantial gains in crop establishment have been realized by pre-planting treatment of seed pieces with fungicides to enhance germination. Specifically, organo-mercurial compounds like Aretan and Agallol, when employed at a concentration of 0.25% for a brief 5–10-minute immersion, exhibit efficacy in eradicating superficial inoculum, albeit with lesser effectiveness against deeply entrenched mycelial structures. Furthermore, specific antifungal agents such as thiophanate methyl, marketed as Topsin-M, display selectively in targeting the red rot pathogen *C. falcatum*, thus presenting a promising avenue for disease management within the agricultural domain (Satyavir, 2003). *In-vitro* studies have demonstrated the complete inhibition of *C. falcatum* growth through chemical control methods, with compounds like Benomyl 50 WP, Folicar and Radomil 75 WP, administered at concentration between 5-50 µg/ml, proving highly effective in suppression of fungal growth (Subhani *et al.,* 2008). Similarly, Bavistin has been found to entirely inhibit *C. falcatum* mycelial growth in laboratory conditions (Nikhil and Sahu, 2014). However, the efficacy of these chemical interventions in field applications remains unconfirmed. Field settings, the primary control of red rot originating from setts, is typically addressed through sett treatment, where the use of fungicides is limited to this specific stage. Treating infected setts with carbendazim and benomyl for 30-60 minutes shows promise to potentially reduce red rot incidence (Malathi and Viswanathan, 2013), though field validation of these methods is essential to assess their real-world effectiveness.

Biological control

Among the manifold strategies employed for the management of soil-borne diseases, biological control emerges as the preeminent choice for ensuring long-term sustainability and effective disease management (Qasim, 2023). The combination of various biological control agents with cultural techniques, soil solarization, fungicides, and the cultivation of disease-resistant crop varieties has been recognized as a potent strategy for effectively managing diseases in a wide range of crop species. Various bio-control agents, including plants growth promoting rhizobacteria (PGPR), have been utilized to manage *C. falcatum* in sugarcane. PGPR, located in the sugarcane rhizosphere, not only promote plant growth by enhancing the production of growth supporting metabolites but also inhibit *C. falcatum* (Bhardwaj *et al*., 2017).

Recent studies have identified several bacterial genera, such as Enterobacter, Pseudomonas, Burkholderia, Bacillus, Gluconaceto bacter and Ochrobactrum, which have shown effectiveness in suppressing *C. falcatum* within the sugarcane rhizosphere through both *in-vitro* and *in-vivo* trials (Katiyar *et al*., 2015). In an *in-vivo* study by Patel *et al*., (2019), Ochrobactrum intermedium (TRD 14) effectively controlled the pathogenicity of *C. falcatum* (cfNAV) and boosted sugarcane growth by 8.2%, primarily by increasing stem diameter. In contrast, Acinetobacter sp. (PK9) and Bacillus sp. (RSC 29) showed limited impact on sugarcane stem height and diameter, although they delayed plant drying, especially in the absence of red rot disease. Escherichia sp. (VRE34) exhibited the most promising results, effectively suppressing the disease while also enhancing sugarcane growth.

Trichoderma harzianum, employed as a bio-agent for the management of red rot disease, exerts its efficacy through direct parasitism of *C. falcatum*, resulting in significant reductions in economic losses among susceptible sugarcane varieties. Furthermore, the application of *T. harzianum* enhances cane yield by stimulating biomass germination and shoot growth. This approach not only proves to be environmentally sustainable and cost-effective but also contributes to soil quality improvement (Shivas and Tan, 2009). Additionally, *T. harzianum* includes systemic resistance in treated sugarcane plants, further augmenting its direct antagonistic effects on *C. falcatum*. Notably, when applied at a rate of 20 kg/ha, *T. harzianum* strain Th37 enhances the availability of nitrogen (N) and phosphorus (P) by 27.65% and 44%, respectively. When combined with TMC/salicylic acid (SA), it elevates red rot defense to 78%, and this defense mechanism reaches 86% when combined with metabolites/SA, as opposed to 60% and 71% for defense in isolation (Yadav *et al*., 2008).

Systemic resistance in plants by Trichoderma strains that belong to the plant-growth promoting (PGPR) family can also be generated. Because PGPR strains were not found in stems or leaves, longitudinal separation of *T. harzianum* and *T. viride* and pathogen was proven, implying that PGPR protection against leaf and stem infections is owing to some sort of systemic resistance that may be induced (ISR). Systemic resistance against Colletotrichum was produced by various local Trichoderma strains in susceptible cultivars of sugarcane (Malathi *et al*., (2002).

Phyto-nanotechnology - A revolutionary tool in agriculture

Global food production demand will have to double by 2050 (Hunter *et al*., 2017). Because of climate changes, which are predicted to prolong the dry season and generate greater aggravation in food production cycles in many sensitive areas, this alarming forecast will become even more distressing. As a result, many plant pathologists and agriculturalists are confronted with a challenging task. On the other side, nanotechnology has been offered as an innovative approach in plant pathology to tackle these expanding challenges to plant health and disease management. In the future, advanced nanotechnology could be used to produce vegetable crops that are more resistant to numerous diseases (Qasim *et al*., 2022).

Green synthesis of nanoparticles has been ecofriendly and cost effective for the management of red rot (Ponmurugan *et al*., 2016). Copper nanoparticles have been shown to have antifungal activity against pathogenic fungi (Cioffi *et al*., 2004). Cu nanoparticles demonstrated significant antifungal activity against *C. falcatum* (Kanhed *et al*., 2014). An experiment was conducted to check the antifungal activity of Cu NPs against red rot disease of sugarcane. Cu NPs were used at different concentrations (25, 50, 150, 250, 500 and 1000 ppm). Results showed that Cu NPs at 1000 ppm was most successful in controlling the mycelial growth by 99.78% followed by 93.75% at 500 ppm (Iliger *et al.,* 2021).

CONCLUSION

Sugarcane, a vital crop in Pakistan, faces significant challenges due to diseases that adversely impact its yield. Among these ailments, red rot disease is the most menacing, often referred to as the “Cancer” of sugarcane. While various disease management practices, such as the development of disease-resistant cultivars, utilization of pathogen free seeds, and the application of different fungicides, have been employed to combat this issue, each approach presents its own set of limitations. For instance, newly developed resistant varieties can become susceptible to evolving pathogen races stemming from excessive pesticide use. Pathogen-free seed usage is effective but does not entirely prevent pathogen entry through irrigation channels. Furthermore, certain fungicides, while effective against the disease, have been banned due to their detrimental health effects. Hence, there is an imperative need to devise alternative disease management strategies to curtail the impact of red rot disease on sugarcane yield. The adoption of an Integrated Disease Management (IDM) practices has shown remarkable results, leading to reduction in red rot incidence, enhancements in growth parameters, and improvements in sugarcane quality attributes. Moreover, there is a pressing call for the development of novel approaches like use of nano-pesticides to combat the disease, including further research into induced systemic resistance and systemic acquired resistance mechanisms.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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