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Research Article

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First Record of a Serious Wilt Disease Induced by *Fusarium sacchari* and Root Borer on Sugarcane in Kenana Scheme, Sudan

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ABSTRACT

Background: Wilt of sugarcane was recorded almost 100 years ago and is one of the major fungal diseases affecting cane production and productivity. Many commercial varieties were withdrawn from cultivation due to their susceptibility to the disease in many countries. Even though the disease was recorded long back,

Methods: For the assessment of disease status and varieties susceptibility of wilt disease, an extensive survey was conducted on sugarcane fields of 2020-2021 of Kenana Sugarcane Company. Isolation of the Pathogen was done by two types of culture in Petri dishes containing PDA the other was incubated in a Plotter test. Both segments were incubated at 28±2°. The pathogenicity test was conducted according to Koch's postulate by Dipping inoculation method and the Plug inoculation method.

Results: The result of the survey revealed that the symptoms of the disease appeared clearly in the fields and the result of laboratory isolation indicated that the pure culture of causing pathogen, *Fusarium sacchari* based on detailed culture morphology. Among the insect pests, root borer was found to associate with wilt disease from sugarcane-infected stalks. The result of the pathogenicity test in pot culture under greenhouse conditions using the variety Co 997 with the pathogenic culture of *F. saccharri* induced the same symptoms. And re-isolation of the pathogen is similar to the isolation from fields

Conclusion: We were concluded that the wilt of sugarcane affects cane productivity in Sudan and this is considered as the first record of wilt disease in sugarcane in Sudan. As sugarcane is an important crop, future disease control methods will soon be started to control this important disease.

Key-words: *Fusarium saccharri*, Kenana Sugar Company, Root borer, Sugarcane, Wilt disease

INTRODUCTION

Sugarcane (*Saccharum officinarum*) is grown in the tropics and subtropical regions of the world. It was estimated that sugarcane was cultivated on over 26 million hectares in more than 90 countries with a worldwide harvest of 1.84 billion tons. Sugarcane cultivation is challenged by several biotic and biotic factor. In addition to other abiotic factors like draught, unseasonal floods etc sugarcane suffers from several diseases caused by fungi, bacteria, viruses, nematodes and mycoplasma. Wilt of sugarcane caused by *Fusarium moniliforme* var. sub glutinans cause serious quantitative and qualitative losses, which have negative effects on sugarcane production, as well as in the sugar industries [1]. The most important diseases in Kenana Sugar Company are smut and ratoon stunting disease [2]. For the first time described a stem rot disease in India in sugarcane under the term wilt and noted *Cephalosporium sacchari* as the causal agent. However, recorded stem rot of the basal portion of unwounded sugarcane stem having species of Fusarium is associated with disease in Barbados [3]. An overall 20.22% loss of cane is caused by different disease which worked out to Tk. 250 corers annually. [4] Fungal diseases are one of the major concerns to agricultural production. Out of 40 sugarcane diseases in Bangladesh, wilt is considered as one of the most damaging [5]. Although there were report on the disease incidence [6] made detailed surveys

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in all the sugarcane growing areas and they reported that the disease severity is highest in Gujarat state, east coastal regions in Tamil Nadu, Andhra Pradesh, and Orissa in tropical India. No definite role of nematodes in stimulating the wilt syndrome in sugarcane is established. However, Viswanathan *et al.* [7] reported a possible association between nematode and wilt fungi. They recorded highest (65%) wilt incidence and reduction in fresh plant weight when sugarcane plants were inoculated with nematodes and wilt fungi (*Fusarium sacchari* and *Acremonium implicatum)* together. Detailed studies have been carried out on phenotypic and genotypic variability at this Institute [8]. Phonotypical characterization of the pathogen was done based on growth rate, pigmentation, texture, nature of phialides and conidia produced. Cultural characteristics of 117 isolates have been studied and they were grouped in to three groups based on the growth rate. Based on the mycelium colour, the isolates were categorized into 7 group's viz., white, orange, orange-pink, pink, dark pink, pinkish violet and reddish brown. More than 75% of the isolates showed typical pinkish pigmentation and other cultures exhibited varying shades of pinkish pigmentation [9]. Wilt fungus in association with some insect pests of sugarcane, particularly stalk borer and scale insects, causes significant damage to the crop. In association with stalk borer, the disease has been reported to bring a loss of about 8.75 tons/ha [10]. Although the disease affects the crop during different phases viz., germination, young crop and maturity, disease expression/symptom are well known in mature crops as yellowing followed by drying of foliage and subsequent withering of infected plants. The germinating setts or germinated settlings also show the disease symptoms and usually, such cases are ignored [11]. Apart from separate infections of *F. sacchari*, combined infections of *F. sacchari* and *Colletotrichum falcatum* causing red rot is found under field conditions in different states in India [12] or *Ceratocystis paradoxa* cause much more damage than when present alone [13]. Found synergistic effect of combined inoculation in disease susceptible clones. Fusarium produced a mild infection, which did not extend beyond the inoculated internode even after three months. In continuation with *C. falcatum*, however, it led to severe infection in all the varieties and the advance of the lesion is greater in the combined infection than infection by *C. falcatum* alone. Between the two pathogens, red rot caused greater deterioration in the quality of sugarcane than wilt [14]. The maximum inhibition zones of the pathogen causing wilt and root rot disease of sugarcane were recorded by Acetobacter, PSB and Trichoderma. Similar results were recorded by Sabalpara *et al.* [15] noticed that for the purpose of controlling sugarcane wilt and root rot pathogen and to obtained efficient isolates, of Trichoderma were tested. The results indicate that all the tested isolates were effective. [16] Reported that the study showed that the different Trichoderma isolates have good antagonistic effect on the mycelium growth of *Fusarium* sp. and *Pythium* sp. These results are in accordance with earlier investigation. The objective of this manuscript is to investigate the presence of wilt disease in sugarcane and early detection.

MATERIALS AND METHODS

To assess the disease status and varieties susceptibility of wilt disease, an extensive survey was conducted on sugarcane fields 2020–2021 of Kenana Sugarcane Company located between latitudes 13:10–12.35^0 North and longitudes 32:40–32:55^0 East, it is about 410 meters above sea level, within the tropic semi/arid climatic zone. A photo was taken from effected stalk also random sample was taken for further investigation. During the survey.

Isolation of the Pathogen- The infected stalks cut into 3 to 4 cm long were surface sterilized by sodium hypochlorite solution 4% for 1 to 2 minutes, washed four times with sterilized distilled water to remove sodium hypochlorite solution and left to dry. Then the sample was divided into two subsamples. Six segments from the subsample were incubated in each Petri dish containing PDA. Similar segments were incubated in the Plotter test. Both segments were incubated at 28±2°C temperature and inspected daily for fungi growth.

Pathogenicity Test- Koch's postulates, firstly the cultivars used in the pathogenicity test should be identical to those on which the disease has been observed and isolated from the field. Then, when the cultures were inoculated into susceptible plants, they must initiate the characteristic disease symptoms. Finally, the organisms were re-isolated in pure culture and re-identified, after which it must be similar to the original organism that had been observed [14] before each step is followed correctly

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and if produced the identical pathogen after re-isolation, then the pathogenicity test had been succeeded [14] the pathogenicity test was done by two methods described as the following:

Dipping inoculation method- Twenty setts from a nursery of sugarcane variety Co 997 was catted as single bud washed by running tab water and then divided into two group ten setts dipped in spore's suspension concentrated $3x10^5$ for ten mints and ten setts dipped in sterile distilled water for ten mints and then planted in a plastic bug in the greenhouse for further investigation. Observation of the disease development was started after one month up to six months of inoculation and at six-month, inoculation stalks of the cane were cut and split open to investigate the internal symptoms [15].

Plug inoculation method- In the plug method, the inoculum was placed in the bore hole and sealed with modelling parafilm to avoid aerial contamination ten cane (stalks) were inoculated in the internode number three from the base of the stalks beside the land, spores' suspension as concentration $3x10^5$, about 1 ml of conidial suspension was placed in the node. Ten stalks as control were treated with sterile distilled water. Observation of the disease development was started after two weeks up to six months of inoculation, inoculation stalks from treated and untreated cane were cut and split open for final observation [16].

RESULTS

The field surveys were conducted in Kenana Sugar Company (KSC) to investigate the occurrence of wilt disease in sugarcane. The wilt stalks were observed dry from the underside part of the stalks and spread upward, when splitting stalks longitudinally the internal symptoms were pith tissue, light to dark purplish-brown. Varying shades of pinkish-red or brownish-red discolouration were seen in the internodes. Stunted with yellowing and withering of crown leaves. The midribs of all leaves in a crown generally turn yellow, while the leaf lamina remains green. The leaves dry up and stalks develop hollowness in the core. The core shows reddish discolouration with longitudinal red streaks passing from one internode to another. In severe cases, spindle-shaped cavities tapering towards the nodes develop in each internode. The canes emit a disagreeable, with a lot of mycelia threads of the fungus covering the cavity (Fig. 1). The culture appears in pure hairy white mycelium (Fig. 2), and the pathogen was identified as *F. sacchari* based on microscopic features. Morphological features of the fungus: the mycelium of the fungus was white cottony, with culture pigmentation peach salmon, vinaceous purple violet, macroconidia general morphology relatively slender, slightly falcate and thin-walled, and number of septa: Usually 3-4 septate. Macroconidia shape and septation: oval, slender and primarily 0-septate, although 1- or 2-septate elongated. Chlamydospores, absent Fig. 3 and also the result of the survey indicated that the wilt disease is associated with root borer as shown in Fig. 4.

Fig. 1: Infected Stalks of Sugarcane by Wilt Disease

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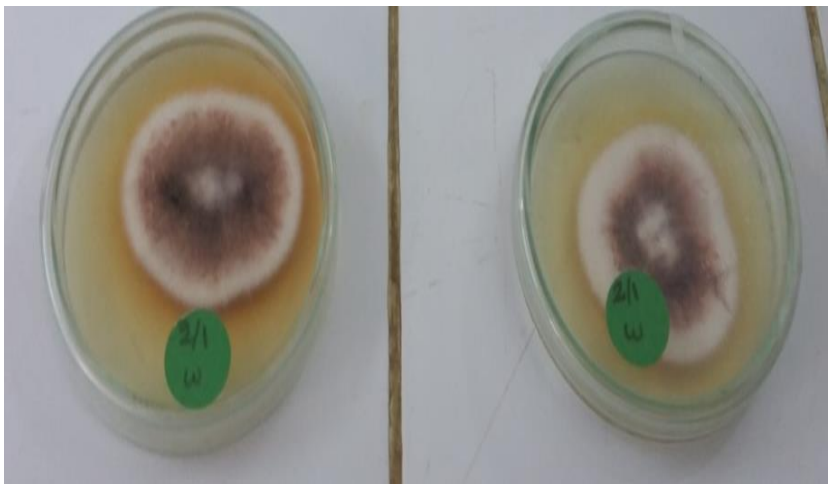

Fig. 2: White Hairy Mycelium of *Fusarium sacchari*

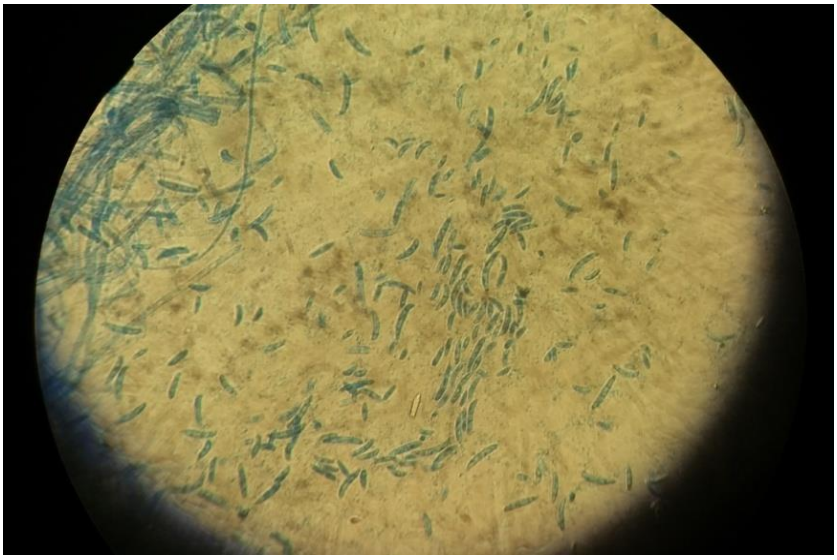

Fig. 3: Macro and Microconidia of *Fusarium sacchari*

Fig. 4: Root borer associated with wilt disease on sugarcane root

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Pathogenicity test- The pathogenicity test was conducted according to Koch's postulates, where the steps are shown in Fig. 5 as followed:

Step A shows the infected stalks collected from sugarcane fields in Kenana State showed symptoms of wilt disease.

Step B showed that the fungi isolated from infected grass in step A grew in culture.

Step C infection of *Fusarium* sp, the result shows typical symptoms as shown in step A.

Step D the results of re-isolation of the fungi from inoculated healthy stalks in step C the pathogens showed the typical characteristic of the isolated fungi from infected sugarcane stalks (the spores and mycelia of species) as shown in the following.

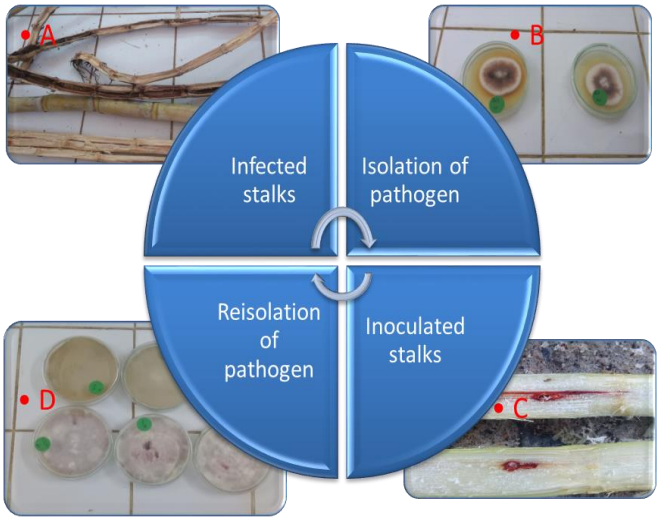

Fig. 5: Pathogenicity Test Cycle According to Koch's Postulates

DISCUSSION

The survey of commercial sugarcane fields in KSC revealed that the wilt disease of sugarcane was present in the variety Co 997 according to the result of the plant's pathology laboratory and confirmed by pathogenicity test, the main causes of this disease according to the test is *F. sacchari*. Also, some insect pest was found associated with wilt disease as root borer. Butler and Khan [3] studied wilt in detail and described *Cephalosporium sacchari* as the associated pathogen. Subsequently, several workers reported *Fusarium moniliforme* var sub-glutinans as the causative pathogen. [5] Coined a new species *F. sacchari.* To which both *C. sacchari* and *F. moniliforme* var. subglutinans were made synonyms. Later [17] distinguished two varieties of *F. sacchari* namely, *F. sacchari* var. sacchari and *F. sacchari* var. sub-glutinans, the former having mostly septate conidia in the aerial mycelium and without sporodochia, while the latter with 1-3 septate conidia, macroconidia more commonly formed in sporodochia. Similar to many vascular wilts in other crops, the wilt of sugarcane is a major disease affecting cane productivity in India. The survey for wilt incidence across the country revealed that the pathogen is widespread in the country [18-20]. Although the disease was reported a century ago. Available information on the associated pathogen is scanty, shallow, and contradictory; hence a detailed investigation was carried out to characterize the pathogenic isolates by cultural, morphological and molecular methods after a comprehensive survey of the disease in major sugarcane growing states. The pathogen exhibited enormous variation in cultural characters and that could not be used to characterize the isolates. Using morphological features and molecular profiles in ISSR and IGSRFLP, the pathogen was identified as *F. sacchari*. Wilt fungus in association with some insect pests of sugarcane, particularly stalk borer and scale insects, causes significant damage to the crop. In association with stalk borer, the disease has been reported to bring a loss of about 8.75 tonnes/ Viswanathan [21] and Viswanathan and Rao [22] reported that the weight decline was 24.9% when the mean incidence of stalk borer-wilt complex was 51.4% [23]. Reported a high incidence of wilt (90%) in association with stalk borer in the cv Co 1148 and the crop was almost unfit for milling. With conservative estimates of a loss of 3-6 tonnes/ha, the disease may cause an annual loss of 12.7-25.40 million tonnes in different years. Hence, the loss to sugarcane production would be between Rs. 1250-2500 crores per annum [24]. The loss in production is borne by both the farmers and the sugar industry. The impact of wilt-infected canes on recoverable sugar in the mill is not assessed properly and sugar mills experience these unaccounted losses every year. In recent years, the sudden outbreak of pokkah boeng across the country was noticed in several varieties [25]. It was found that *Fusarium* sp. associated with pokkah boeng also caused stalk infections and produced wilt in certain varieties. It is expected that further studies in this area would bring a new dimension to the Fusaria associated with pokkah boeng and wilt. *Eldana saccharina* is associated with Fusarium species in maize [26] and sugarcane [27]. In maize, endophytic colonization by *F. verticilliodes* was

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correlated with higher *E. saccharina* infestation and damage compared with plants treated with fungicide, suggesting a beneficial relationship between the fungus and the insect [26]. In sugarcane, *E. saccharina* damage is associated with infection by *Fusarium* sp. where larval borings facilitate access of the fungus to the inner stalk resulting in the rot of tissue surrounding the insect borings [27].

CONCLUSIONS

Wilt disease symptoms in sugarcane are very common in the variety Co 997 in Kenana Sugar Company-Sudan. Several investigations revealed the occurrence of wilt disease induced by *F. sacchari* was responsible for wilting of sugarcane stalks and this disease was associated with root borer mix infection leading to dryness of the whole stalk. Similar to many vascular wilts in other crops, the wilt of sugarcane is a major disease affecting cane productivity in Sudan and this is considered the first record of wilt disease in sugarcane in Sudan.

The survey for wilt disease in Kenana Sugar Company revealed that the stalks rot of basal portion of unwounded stalks. As sugarcane is an important cash crop in the Sudan, future disease control methods will soon be started to control this important disease.

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Data collection- Hawa Ahmed Ibrahim, Abu Bakr Abdalla Ali, Elnaeem Mohamed and Elnair Akasha Mohammed
Data analysis and interpretation- Elnair Akasha Mohammed
Literature search- Elnair Akasha Mohammed
Writing article- Elnair Akasha Mohammed
Critical review- Prof.Ahmed Hashim Ahmed
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Final approval- Prof.Ahmed Hashim Ahmed

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