Occurrence of Anthracnose Disease of Turkey Berry (*Solanum torvum*) at Bunso, Eastern Region, Ghana.

ABSTRACT

Aims: To identify the causal agent of anthracnose disease of *Solanum torvum*, determine whether the pathogen is seedborne and also to ascertain the cross infection potential of the pathogen on other *Solanaceous* crops of economic importance.

Place and Duration of Study: Bunso, in the East Akim District of the Eastern Region of Ghana, between January and October, 2017.

Methodology: The disease symptoms on matured fruits, leaves, stems and flowers of *Solanum torvum* were carefully observed for documentation with magnifying glasses and the naked eyes. The pathogen was Isolated on PDA after incubation for five days and the Identification was based on the colony, morphology and conidial characteristics with reference to laboratory manuals. The virulence of the fungal isolates from the *S. torvum* fruits was determined through pathogenicity tests. A seed health test was conducted in accordance with the International Seed Testing Association (ISTA) to determine whether the pathogen is seedborne. Conidial suspension of *Colletotrichum acutatum* (1×10^3 per ml) was used to inoculate pepper and eggplant fruits in a cross infectivity test.

Results: *Colletotrichum acutatum* was repeatedly isolated and identified as the causal agent of the disease on the fruits and was also seedborne. In cross infectivity studies, the pathogen produced characteristic anthracnose symptoms on both eggplant and pepper which happens to belong to the same Solanaceae family just as the turkey berry.

Conclusion: The anthracnose disease of *Solanum torvum* at Bunso, in the Eastern Region of Ghana is caused by *Colletotrichum acutatum* that has the potential to cross infect other *Solanaceous* species. This study is the first scientific report of the occurrence of anthracnose disease of *S. torvum* in Ghana.

Keywords: *Anthracnose; Seedborne; Colletotrichum acutatum; Cross infection; Solanaceae.*

1. INTRODUCTION

Solanum torvum Sw. (Turkey berry) is an erect, prickly perennial shrub that is usually one to three meters high and belongs to the *Solanaceae* family. The plant is native and cultivated in Africa and the West Indies [1].

In Ghana, it is known locally as “Nsusuaa” or “Beduro” among other names and is often found in home gardens for use as fruit vegetable and medicine. The edible immature fruits are commonly sold as a vegetable in the Southern parts of Ghana. They are mostly patronized as an important ingredient for soups and stews to boost one’s haemoglobin level.

Solanum torvum is a valuable source of medicinally important compounds and its edible fruits are a store house of minerals, vitamins, antioxidants and other nutrients [2]. Many pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant activity, cardiovascular, immunomodulatory and nephroprotective activity supporting its traditional uses [2,3,4,5]. *Solanum torvum* has also been found to contain all the electrolytes that are constituents of blood plasma (including K, Ca and Mg) and therefore serves as a good source of iron for haemoglobin production [6].

Solanum torvum is extensively used in breeding as a rootstock for eggplant and tomatoes to confer resistance to bacterial wilt, Phomopsis fruit rot and nematodes [7,8]. *Solanum torvum* hardly succumbs to diseases and this supports its use as a source of resistant gene transfer.

However, recently some *Solanum torvum* plants at Bunso in the East Akim District of the Eastern Region of Ghana have been observed to show symptoms of brown lesions on the fruit with a mean incidence of 60 %. These lesions are suspected to be incited by a fungus. A study was therefore initiated to identify the causal agent, determine whether the pathogen is seedborne and also to ascertain the cross infection potential of the pathogen on other *Solanaceous* crops of economic importance.

1. MATERIALS AND METHODS

2.1 Observation of disease symptoms: The disease symptoms on matured fruits, leaves, stems and flowers of *Solanum torvum* were carefully observed for documentation with magnifying glasses and the naked eyes.

2.2 Isolation and Identification of Pathogen: Samples of *S. torvum* fruits with the brown lesions were collected from various fields at Bunso for laboratory studies at the CSIR-Plant

Genetic Resources Research Institute, Bunso. The study area is characterized by a bimodal rainfall pattern with an annual mean rainfall of 1500 mm and mean temperature of 27 ℃. Small pieces of tissues of about 2 mm in size were cut from the periphery of the diseased portions of the fruits with a sterilized scalpel. The cut tissues were surface sterilized in 5 % sodium hypochlorite solution for one minute and rinsed three times in sterile distilled water. These samples were blotted dry on sterile blotter papers in a laminar flow cabinet and plated on Potato Dextrose Agar (PDA) in 90 mm diameter sterilized Petri dishes. The plates were incubated at 28 ℃ in an incubation chamber for five days. The developing fungal colonies were subcultured on fresh PDA plates to obtain pure cultures. The subcultured plates were turned upside down to enhance mycelial growth. The fungal isolates were identified based on their colony, morphology and conidial characteristics with reference to laboratory manuals developed by [9] and [10]

2.3 Pathogenicity Test: The virulence of the fungal isolates from the *S. torvum* fruits was determined through pathogenicity tests. Fresh, healthy fruits of a growing plant were surface sterilized using absolute ethanol. Conidial suspension of the two fungi isolated served as the inoculum for the inoculation. Conidia were harvested from 7 day old cultures by flooding petri dishes with 5 ml of sterile distilled water and dislodging conidia by gently scraping colonies with a glass rod. Conidial suspensions were then filtered through sterile cheesecloth. The two inocula were adjusted with sterile distilled water to a concentration of 1×10^3 per ml (11). Conidial suspension of approximately 0.5 ml was injected 2-3 mm deep into the skin of the surface sterilized healthy fruits on the growing turkey berry plants with a sterile syringe. Sterile distilled water was injected into other healthy fruits on the plants as the control treatment. The inoculated fruits were wrapped with transparent polyethylene bags to create a humid environment for disease development.

To fulfill Koch's postulates, re-isolation of the fungus was made from fruits that showed symptoms similar to those observed in the field.

2.4 Determination of whether the pathogen is seedborne: A seed health test was conducted in accordance with the International Seed Testing Association [12] to determine whether the pathogen is seedborne. Four hundred seeds randomly selected from a bulk of seeds of *S. torvum* (landrace) were assessed for seedborne fungi. The seeds were plated in sterile petri dishes lined with three layers of moistened blotter papers to provide enough moisture for the seeds. Twenty five seeds were plated per petri dish. The plated seeds were incubated at 28℃ for seven days under alternating 12 hours light and 12 hours darkness. After the seven (7) days

of incubation, the seeds were examined under a stereo microscope for the identification of seedborne fungi.

2.5 Cross infection status of the pathogen: The disease causal organism identified was assessed for its ability to cross infect pepper and eggplant fruits which also are of the *Solanaceae* family. Conidial suspension of *Colletotrichum acutatum* was used to inoculate the pepper and eggplant fruits in the same manner as in the pathogenicity test. Regular observations were made daily for the characteristic anthracnose symptom development and when symptoms did occur; re-isolations were made in accordance with Koch's postulates.

3. RESULTS

3.1 Symptomatology: Infected Turkey Berry fruits showed sunken dark brown necrotic lesions circular to irregular in shape. The lesion started as a small dark spot that enlarged as the disease progressed and covered a much bigger necrotic area (Plate 1). Brown conidial masses were seen either in concentric rings or scattered in the necrotic areas of the fruits. The disease affected the fruits at all stages; from the bud stage to the mature fruit stage (Plate 2). In advanced stages of the disease, the necrotic areas became very dry and hard whilst the healthy portions remained fresh and intact but eventually the entire fruit became rotten and mummified.

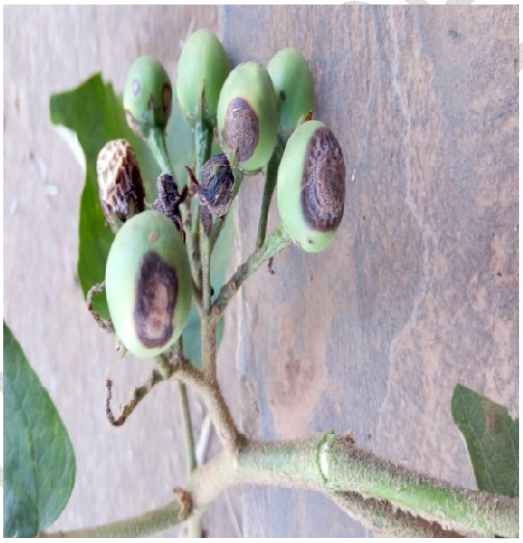

Plate 1: Disease symptoms on mature fruits

Plate 2: Disease symptoms on immature fruits and flower buds

3.2 The causal pathogen: Two fungal species belonging to two genera were isolated and identified. These fungi were *Colletotrichum acutatum* (Plate 3 & 4) and *Curvularia pallescens* (Plate 5 & 6)

Plate 3: Culture plate of *Colletotrichum acutatum*

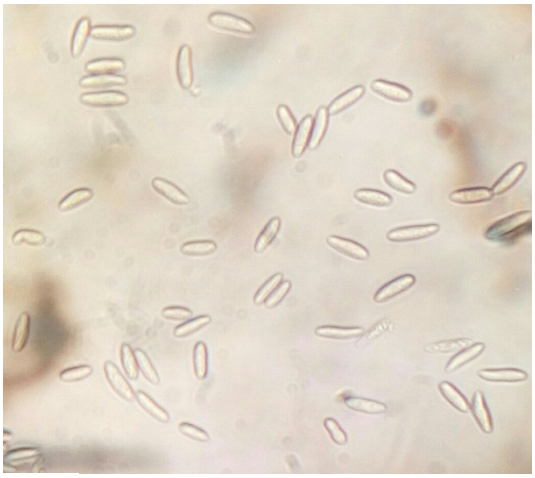

Plate 4: Conidia of *Colletotrichum acutatum*

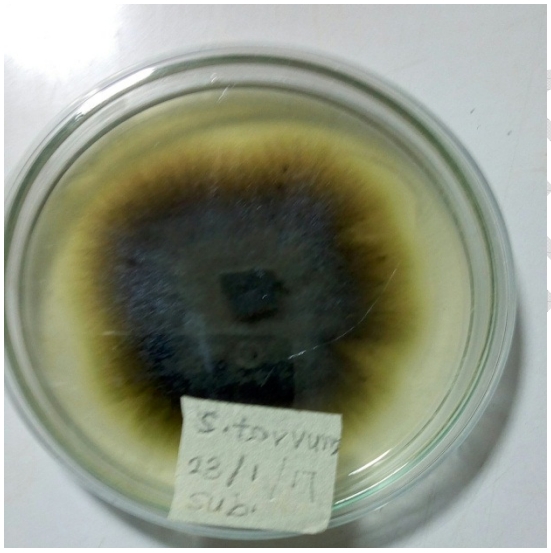

Plate 5: Culture plate of *Curvularia pallescens*

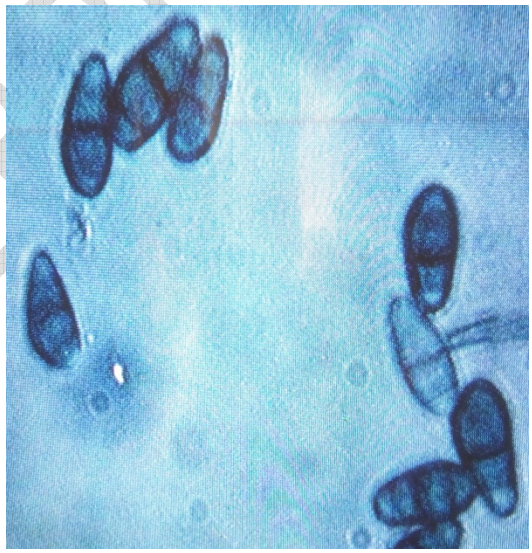

Plate 6: Conidia of *Curvularia pallescens*

Colletotrichum acutatum showed a whitish colony colour on PDA. The conidia were hyaline, straight, fusiform, single celled and acute or pointed at one or both ends. The length of the conidia ranged from 7.5 – 10.4µm and width of 2.7 – 4.2 µm. *Curvularia pallescens* colony colour was black with straight and curved conidia with two to three septation.

3.3 Pathogenicity Test: Anthracnose symptoms similar to those observed on the original diseased turkey berry fruits developed on the fruits inoculated with *Colletotrichum acutatum*

seven days after inoculation. The fungus was repeatedly re-isolated from the fruits in accordance with the Koch's postulate. For the control, the fruits inoculated with sterile distilled water remained healthy.

However, for *Curvularia pallescens*, the inoculated fruits showed no symptoms at all and the fruits remained healthy. *Colletotrichum acutatum* was identified as the pathogen of anthracnose disease of *Solanum torvum.*

3.4 Determination of whether the pathogen is seedborne: The surface sterilized seeds of *S. torvum* did not record any incidence of seedborne fungus. However, the pathogen, *Colletotrichum acutatum* was isolated from the plated unsterilized seeds seven days after incubation. The infected seeds were subsequently plated on PDA for proper growth and identification as shown on plate 7.

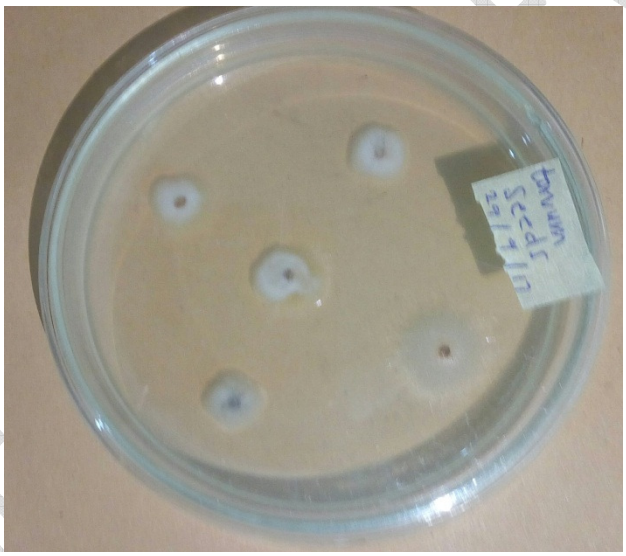

Plate 7: *C. acutatum* growth on *S. torvum* seeds plated on PDA

3.5 Cross infection status of the pathogen: *Colletotrichum acutatum* isolated from the diseased fruits of *S. torvum* was assessed for its potential to cross infect eggplant and pepper fruits by artificial inoculations. Both the artificially inoculated eggplant and pepper fruits on the growing plants expressed the characteristic symptoms of anthracnose (Plate 8 & 9). *Colletotrichum acutatum* was repeatedly re-isolated from the inoculated fruits just as in the pathogenicity test. The control fruits on the other hand, remained healthy.

Plate 8: Necrotic brown lesions on the artificially inoculated eggplant fruits

Plate 9: Necrotic brown lesions on the artificially inoculated pepper fruit

4. DISCUSSIONS

This study identified the causal agent of the brown lesion symptoms of *Solanum torvum* fruits; ascertained whether the disease pathogen is seedborne and determined the cross infection potential of the disease pathogen on other *Solanaceous* species of economic importance. The brown lesion disease symptoms observed on *Solanum torvum* plant did not differ from that of anthracnose diseases of other known crops. The only unique symptom of the disease observed was the fact that the symptoms occurred at all the stages of the *Solanum torvum* fruit, even at the flower bud formation stage to mature fruit stage. This characteristic of the disease on *S. torvum* makes it very devastating, in that, it can lead to a gradual extinction of the species since fruits and viable seeds may not be produced for dispersal. Bud infection to fruit rot caused by anthracnose pathogens has been reported on blueberry fruits [13].

From the pathogenicity test, it is certain that, *Colletotrichum acutatum* is the causal organism of the anthracnose disease of *Solanum torvum. Colletotrichum* species has been reported to cause anthracnose disease of other berries such as blueberry and strawberry [14, 15]. This study is the first scientific report of the occurrence of anthracnose disease of *S. torvum* in Ghana.

Colletotrichum species are broad-range pathogens; many species can infect a single host, and single species can infect diverse hosts [16]. However, in this study, *C. acutatum* was the only species isolated from all the diseased fruit samples collected from various fields. Therefore, *Colletotrichum acutatum* is the only known causal organism for the anthracnose disease of *Solanum torvum* fruits at Bunso, Ghana.

The surface sterilized seeds did not contain any seedborne pathogen. However, the unsterilized seeds of *Solanum torvum* harboured the pathogen, *Colletotrichum acutatum.* This indicates that the pathogen of anthracnose of *S. torvum* is seed borne. The absence of *C. acutatum* propagules on the sterilized seeds reveals the external nature of the pathogen on the seeds.

Different species of *Colletotrichum* has been reported to have the potential to cross infect a variety of fruits. For instance, in Israel, it was revealed that *C. gloeosporioides* isolates from almond, apple, avocado and mango as well as *C. acutatum* isolates from anemone, apple, peach and strawberry infected detached fruits from different hosts [15,17].

Colletotrichum acutatum from strawberry only survived on pepper, eggplant, tomato, bean and weed species without causing disease symptoms [18], however, in the current study, *C. acutatum* isolated from the *S. torvum* fruits produced the characteristic symptoms of anthracnose on both eggplant and pepper fruits. Although this was obtained through artificial inoculation, it indicates that, *C. acutatum* from *Solanum torvum* has the potential to cross infect eggplant and pepper fruits.

The cross infection potential of *C. acutatum* isolates of *S. torvum* on eggplant and pepper can be attributed to the fact that these crop species have similar genetic characteristics, because they belong to the same family, *Solanaceae*. The result from the cross infectivity studies, suggests that, *C. acutatum* isolates from *S. torvum* fruits are not host specific due to their ability to cross infect eggplant and pepper fruits. This corroborates the assertion that, *C. acutatum* has a broad host range, and it is relatively non-specific [19].

According to [19], *C. acutatum* from strawberry could parasitize and cause disease on other hosts or, alternatively, survive on other crops and on weeds without producing symptoms. Similarly, the results from the current study, suggest that the *S. torvum* plants which are mostly found growing in the wild, around cultivated fields could serve as an alternative host and source of inoculum for other *Solanaceous* species such as pepper, tomato and eggplant and cause anthracnose diseases.

5. CONCLUSION

The results from this study has revealed that, the anthracnose disease of *Solanum torvum* at Bunso, in the Eastern Region of Ghana is caused by *Colletotrichum acutatum* that has the potential to cross infect other *Solanaceous* species such as pepper and eggplant. The occurrence of the disease on *Solanum torvum*, an important vegetable and medicinal plant as well as a great source of disease resistant gene for breeding crops is a wake up call for a conscious effort to be made to cultivate *S. torvum* in home gardens and farms. This will ensure the perpetuation of the species and prevent imminent extinction.

COMPETING INTEREST

Authors have declared that no competing interest exists.

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