

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

3
4 **ABSTRACT**

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

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

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

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

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

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

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30 1. INTRODUCTION

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

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

38 *Solanum torvum* is a valuable source of medicinally important compounds and its edible fruits
39 are a store house of minerals, vitamins, antioxidants and other nutrients [2]. Many
40 pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant activity,
41 cardiovascular, immunomodulatory and nephroprotective activity supporting its traditional uses
42 [2,3,4,5]. *Solanum torvum* has also been found to contain all the electrolytes that are
43 constituents of blood plasma (including K, Ca and Mg) and therefore serves as a good source of
44 iron for haemoglobin production [6].

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

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

54 1. MATERIALS AND METHODS

55 **2.1 Observation of disease symptoms:** The disease symptoms on matured fruits, leaves,
56 stems and flowers of *Solanum torvum* were carefully observed for documentation with
57 magnifying glasses and the naked eyes.

58 **2.2 Isolation and Identification of Pathogen:** Samples of *S. torvum* fruits with the brown
59 lesions were collected from various fields at Bunso for laboratory studies at the CSIR-Plant

60 Genetic Resources Research Institute, Bunso. The study area is characterized by a bimodal
61 rainfall pattern with an annual mean rainfall of 1500 mm and mean temperature of 27 °C. Small
62 pieces of tissues of about 2 mm in size were cut from the periphery of the diseased portions of
63 the fruits with a sterilized scalpel. The cut tissues were surface sterilized in 5 % sodium
64 hypochlorite solution for one minute and rinsed three times in sterile distilled water. These
65 samples were blotted dry on sterile blotter papers in a laminar flow cabinet and plated on Potato
66 Dextrose Agar (PDA) in 90 mm diameter sterilized Petri dishes. The plates were incubated at 28
67 °C in an incubation chamber for five days. The developing fungal colonies were subcultured on
68 fresh PDA plates to obtain pure cultures. The subcultured plates were turned upside down to
69 enhance mycelial growth. The fungal isolates were identified based on their colony, morphology
70 and conidial characteristics with reference to laboratory manuals developed by [9] and [10]

71 **2.3 Pathogenicity Test:** The virulence of the fungal isolates from the *S. torvum* fruits was
72 determined through pathogenicity tests. Fresh, healthy fruits of a growing plant were surface
73 sterilized using absolute ethanol. Conidial suspension of the two fungi isolated served as the
74 inoculum for the inoculation. Conidia were harvested from 7 day old cultures by flooding petri
75 dishes with 5 ml of sterile distilled water and dislodging conidia by gently scraping colonies with
76 a glass rod. Conidial suspensions were then filtered through sterile cheesecloth. The two
77 inocula were adjusted with sterile distilled water to a concentration of 1×10^3 per ml (11). Conidial
78 suspension of approximately 0.5 ml was injected 2-3 mm deep into the skin of the surface
79 sterilized healthy fruits on the growing turkey berry plants with a sterile syringe. Sterile distilled
80 water was injected into other healthy fruits on the plants as the control treatment. The inoculated
81 fruits were wrapped with transparent polyethylene bags to create a humid environment for
82 disease development.

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

85 **2.4 Determination of whether the pathogen is seedborne:** A seed health test was conducted
86 in accordance with the International Seed Testing Association [12] to determine whether the
87 pathogen is seedborne. Four hundred seeds randomly selected from a bulk of seeds of *S.*
88 *torvum* (landrace) were assessed for seedborne fungi. The seeds were plated in sterile petri
89 dishes lined with three layers of moistened blotter papers to provide enough moisture for the
90 seeds. Twenty five seeds were plated per petri dish. The plated seeds were incubated at 28°C
91 for seven days under alternating 12 hours light and 12 hours darkness. After the seven (7) days

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

94 **2.5 Cross infection status of the pathogen:** The disease causal organism identified was
95 assessed for its ability to cross infect pepper and eggplant fruits which also are of the
96 *Solanaceae* family. Conidial suspension of *Colletotrichum acutatum* was used to inoculate the
97 pepper and eggplant fruits in the same manner as in the pathogenicity test. Regular
98 observations were made daily for the characteristic anthracnose symptom development and
99 when symptoms did occur; re-isolations were made in accordance with Koch's postulates.

100 3. RESULTS

101 **3.1 Symptomatology: Infected Turkey Berry fruits** showed sunken dark brown necrotic lesions
102 circular to irregular in shape. The lesion started as a small dark spot that enlarged as the
103 disease progressed and covered a much bigger necrotic area (Plate 1). Brown conidial masses
104 were seen either in concentric rings or scattered in the necrotic areas of the fruits. The disease
105 affected the fruits at all stages; from the bud stage to the mature fruit stage (Plate 2). In
106 advanced stages of the disease, the necrotic areas became very dry and hard whilst the healthy
107 portions remained fresh and intact but eventually the entire fruit became rotten and mummified.



108 Plate 1: Disease symptoms on mature fruits



109 Plate 2: Disease symptoms on immature fruits
and flower buds

110 **3.2 The causal pathogen:** Two fungal species belonging to two genera were isolated and
111 identified. These fungi were *Colletotrichum acutatum* (Plate 3 & 4) and *Curvularia pallescens*
112 (Plate 5 & 6)



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Plate 3: Culture plate of *Colletotrichum acutatum*

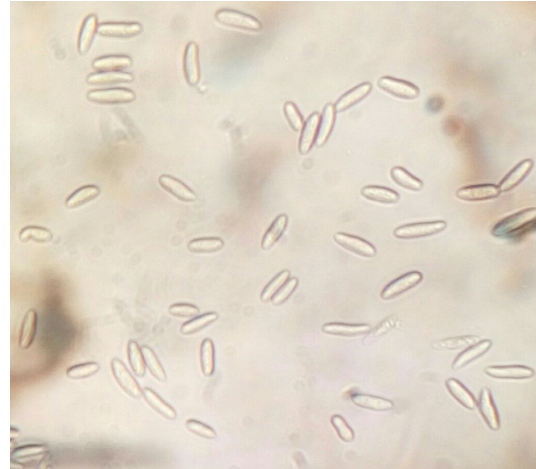
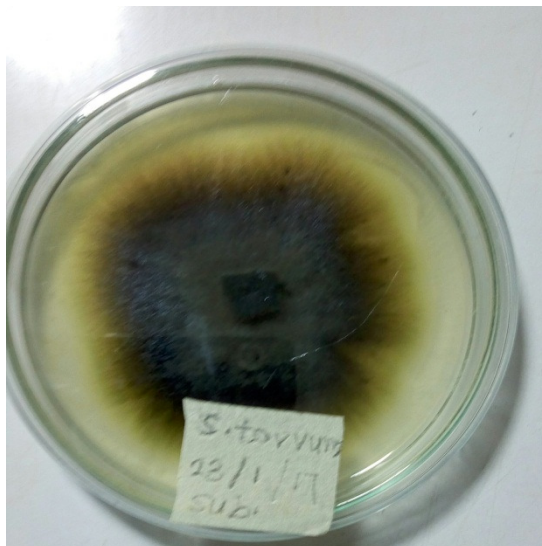


Plate 4: Conidia of *Colletotrichum acutatum*



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Plate 5: Culture plate of *Curvularia pallescens*



Plate 6: Conidia of *Curvularia pallescens*

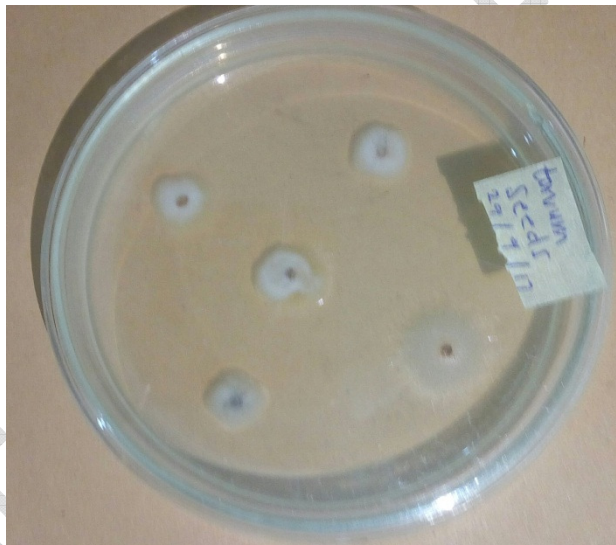
118 *Colletotrichum acutatum* showed a whitish colony colour on PDA. The conidia were hyaline,
119 straight, fusiform, single celled and acute or pointed at one or both ends. The length of the
120 conidia ranged from 7.5 – 10.4µm and width of 2.7 – 4.2 µm. *Curvularia pallescens* colony
121 colour was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

122 **3.3 Pathogenicity Test:** Anthracnose symptoms similar to those observed on the original
123 diseased turkey berry fruits developed on the fruits inoculated with *Colletotrichum acutatum*

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

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

130 **3.4 Determination of whether the pathogen is seedborne:** The surface sterilized seeds of *S.*
131 *torvum* did not record any incidence of seedborne fungus. However, the pathogen,
132 *Colletotrichum acutatum* was isolated from the plated unsterilized seeds seven days after
133 incubation. The infected seeds were subsequently plated on PDA for proper growth and
134 identification as shown on plate 7.



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

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137 **3.5 Cross infection status of the pathogen:** *Colletotrichum acutatum* isolated from the
138 diseased fruits of *S. torvum* was assessed for its potential to cross infect eggplant and pepper
139 fruits by artificial inoculations. Both the artificially inoculated eggplant and pepper fruits on the
140 growing plants expressed the characteristic symptoms of anthracnose (Plate 8 & 9).
141 *Colletotrichum acutatum* was repeatedly re-isolated from the inoculated fruits just as in the
142 pathogenicity test. The control fruits on the other hand, remained healthy.

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

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

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154 4. DISCUSSIONS

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

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

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

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

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

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

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

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

201 5. CONCLUSION

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

209 COMPETING INTEREST

210 Authors have declared that no competing interest exists.

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212 REFERENCES:

- 213 1. Adjanohoun J, Aboubakar N, Dramane K, Gbile ZO, Kamanyi A. Traditional Medicine and
214 Pharmacopeia contribution to ethanobotanical and floristic studies in Cameroon. In:
215 CNPMS, Porto-Novo, Benin. 1996: 50-52.
- 216 2. Jaiswal BS. *Solanum torvum*: A review of its traditional uses, phytochemistry and
217 pharmacology. *International Journal of Pharma and Biosciences*. 2012; 3 (4):104-111
- 218 3. Mohan M, Jaiswal BS, Kasture S. Effects of *Solanum torvum* on blood pressure and
219 metabolic alterations in fructose hypertensive rats. *Journal of Ethnopharmacology*. 2009;
220 126:86-89
- 221 4. Ghandi CR, Ignacimuthu S, Paulraj MG, Sasikumar P. Antihyperglycemic activity and
222 antidiabetic effect of methyl caffeate isolated from *solanum torvum* Swartz. Fruits in
223 streptozotocin induced induced diabetic rats. *European Journal of Pharmacology*. 2011;
224 309(23):623-631.
- 225 5. George K, Patrick A, Terrick A. Immunomodulatory and erythropoietic effects of aqueous
226 extract of the fruits of *solanum torvum* Swartz.(*Solanaceae*). *Pharmacognosy Research*.
227 2011; 3(2):130-134.
- 228 6. Serfor-Armah Y, Fletcher JJ, Oppong, FK. Determination of some essential elements in
229 *Solanum torvum* using neutron activation analysis. *Journal of Ghana Science Association*.
230 2005; 7(2) 77- 82.

- 231 7. Hebert Y. Comparative resistance of nine Solanum species to bacterial wilt (*Pseudomonas*
232 *solanacearum*) to the nematode *Meloidogyne incognita*. Importance for breeding aubergine
233 (*Solanum melongena* L.) in a humid tropical zone. *Agronomie*. 1985; 5(1): 27-32
- 234 8. Jadari R, Sihachaakr D, Rossignol L, Decreux G, Rouselle P, Bourgeois F. Transfer of
235 resistance to *Verticillium dahliae* from *Solanum torvum* SW. into potato by protoplast
236 electrofusion. Proceeding of the joint conference of the EAPR Breeding and varietal
237 Assessment Section, Landerneau, France; 1992.
- 238 9. Barnett HL, Hunter BB. Illustrated Genera of imperfect Fungi. 4th edition. APS Press, St.
239 Paul, Minnesota; 1998.
- 240 10. Watanabe T. Pictorial atlas of soil and seed fungi morphologies of cultured fungi and key to
241 species. Second edition, CRC Press. Boca Raton, London, New York, Washington DC; 2002.
- 242 11. Akhter S, Alam S, Islam S, Lee W. Identification of fungal pathogen that causes strawberry
243 Anthracnose in Bangladesh and Evaluation of *In vitro* fungicide Activity. *Mycobiology*. 2009;
244 37(2): 77 – 81.
- 245 12. International Seed Testing Association (ISTA). Proceedings of the International Seed
246 Testing Association. International Rules of Seed Testing. *Seed Science and Technology*. 1999;
247 15: 1-9.
- 248 13. Demarsay A and Oudemans, PV. Blueberry anthracnose: From bud infection to fruit rot.
249 (Abstract) *Phytopathology*. 2004; 94(25).
- 250 14. Peres NA, Timmer LW, Adaskaveg JE, Correl JC. Lifestyles of *Colletotrichum acutatum*.
251 *Plant Disease*. 2005; 89 (8):784-796
- 252 15. Freeman S, Katan T, Maymon M, Zveibil A. Genetic diversity within *Colletotrichum*
253 *acutatum sensu* Simmonds. *Phytopathology*. 2001a; 91:586-592.
- 254 16. Freeman S, Katan T, Shabi E. Characterisation of *Colletotrichum* species responsible for
255 anthracnose diseases of various fruits. *Plant Disease* .1998; 85:596-605
- 256 17. Freeman, S Shabi E. Cross infection of subtropical and temperate fruits by *Colletotrichum*
257 species from various hosts. *Physiology and Molecular Plant Pathology*. 1996; 49:395-404
- 258 18. Freeman S. Management, survival strategies and host range of *Colletotrichum acutatum* on
259 strawberry. *Hortscience*. 2008; 43(1).

260 19 . Peres NA, Kuramae EE, Dias MSC, de Souza NL. Identification of *Colletotrichum* spp.
261 affecting fruits after harvest in Brazil. *Journal of phytopathology*. 2002; 150: 128-134.

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UNDER PEER REVIEW