

# First Report of *Stemphylium vesicarium* Causing Onion Stemphylium Leaf Blight in Ethiopia

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## Abstract

A suspected fungal disease was observed on onion leaves during survey in onion fields of the central rift valley of Ethiopia in the offseason in 2019. The disease symptoms were round to oval small spots on onion leaves which coalesce to form blighted leaves that change to brown lesion and black sporulation with time. The disease was of high incidence (up to 95.2%) and severity (up to 4.67) where in some fields it has been causing early plant senescence and reduced bulb size during harvest with massive yield loss, named to be “Yeshinkurt Ebola” to mean “Ebola of onion” by farmers. Dirty white to light grey front colony color and light brown to deep brown reverse colony color with alternative white and brown band fungal growth were isolated from infected onion leaf samples. The conidia were olive brown, oval to ovoid, oblong and ovoid to oblong are born on conidiophores. Pathogenicity of the pathogen was confirmed on 45-days-old onion (cv. Nafis) plant, and it was identified as *Stemphylium vesicarium* based on cultural, morphological and pathogenic results. This is the first report of *S. vesicarium*, the causative agent of onion stemphylium leaf blight.

## Keywords

*Stemphylium vesicarium*, Onion, Prevalence, Severity, Pathogenicity

## 1. Introduction

Many small- and large-scale farmers in the main onion-producing areas of central rift valley of Ethiopia grow onion (*Allium cepa* L.). In total, 31,673 ha of land were covered by onion with 293,887.6 tonnes of production, CSA [1]. Nowadays, the area under onion production is increasing. However, its production and/or productivity is affected by many biotic (diseases, and insects pests) and abiotic like imbalanced fertilizer, uneven irrigation and inappropriate agro-

onomic practices, Amin *et al.* [2]. Currently, a strange disease, expected to be stemphylium leaf blight is observed in farmers' field and research stations of the CRV.

Stemphylium leaf blight caused by *Stemphylium vesicarium* Wallr (Simmons) (teleomorph: *Pleospora allii* (Rabenh.) Ces. and De Not) is one of the most destructive fungal diseases in *Allium* species. The host range of the pathogen varies among different crops such as garlic, Suheri and Price [3], chilli pepper, Vitale *et al.* [4], leek, Suheri and Price [3], asparagus, Foster [5], pear, Llorente and Montesinos [6] and Köhl *et al.* [7].

Stemphylium leaf blight was first identified from India in 1975 on garlic, Rao and Pavgi [8] and has subsequently been recorded in USA, Shishkoff and Lorbeer [9], South Africa, Aveling and Naude [10]; Aveling and Snyman [11], Spain, Basallote *et al.* [12], Brazil, Boiteux *et al.* [13] and Turkey, Polat *et al.* [14]. The genus *Stemphylium* comprises 28 phylogenetically distinguished plant pathogenic species, Woudenberg *et al.* [15]. Recently, Brahamanage *et al.* [16] reviewed the existence of the genus *Stemphylium* in the Netherlands, Bangladesh, Egypt, Nepal, Syria, and China on different host plants. *Stemphylium vesicarium* is considered as very disruptive disease, which can cause up to 90% yield losses on onion, Miller *et al.* [17]; Tomaz and Lima [18]; Lorbeer [19], 60 - 90% yield loss on pear, Llorente and Montesinos [20], and complete spears loss on asparagus, Hausbeck *et al.* [21].

In Ethiopia, purple blotch (*Alternaria porri*) and downy mildew (*Peronospora destructor*) diseases are considered as the major constraints of onion production and productivity, Wondirad *et al.* [22]. Purple blotch is prevalent in all onion-growing areas of the country. It attacks leaves, bulb and seed stalks and subsequently reduces yield and quality. Since 2018, however, a new disease symptom, which confuses with purple blotch disease, was observed in the central rift valley areas of Ethiopia. The disease typically attacks leaf, which starts with round to oval small spots that later coalesce to form blighting to leaf tissue. The lesion turns brown to tan, for some time; the disease was probably misdiagnosed as purple blotch since their symptoms are very similar. Samples submitted to the plant pathology laboratory of Melkassa Agricultural Research Center of Ethiopian Institute of Agricultural Research (EIAR) revealed the presence of stemphylium leaf blight (SLB).

Following the detection of the pathogen (*S. vesicarium*) survey was conducted to have a clear picture of its prevalence, incidence and severity. The survey covered the main onion producing areas in the central rift valley (CRV) of Ethiopia. Cultural and morphological characteristics of symptomatic onion leaf samples of the survey indicated the existence SLB disease (*S. vesicarium*).

## 2. Materials and Methods

### 2.1. Description of the Study Area

The field survey was conducted during the 2019 cropping season. The survey

encompassed a total of 26 onion fields in the five districts (Adama, Bora, Dugda, Jeju and Lumie) of the CRV (located 08°00'10.6" to 08°30'01.7" N, 038°43'53.9" to 039°35'21.8" E) of Ethiopia (**Table 1**). Characteristic features of surveyed onion fields were summarized in **Table 2**.

## 2.2. Sampling and Sampling Units

Fields were ordinarily selected at intervals of 5 - 10 km along the main roads. When necessary, the sample sizes (the number of observed fields per district) and the distance between sample units (the arbitrarily selected quadrants, 5 - 10) per field were adjusted to suit crop distribution and field-size. All sampled fields

**Table 1.** Descriptions of surveyed onion fields and associated disease pressure in the Central Rift Valley of Ethiopia (Jan. 14-18, 2019).

ID	District	Locality	Var.	Alt (masl)	Latitude (N)	Longitude (E)	DAT	DI	DS	YFFO
1	Jeju	HD	BR	1258	08°30'01.7"	039°34'24.1"	75	87.5	4	2017
2	Jeju	HD	BR	1267	08°29'49.1"	039°34'30.1"	90	75	3	2017
3	Jeju	HD	BR	1256	08°29'45.6"	039°34'20.1"	67	72.5	3	2017
4	Jeju	HD	BR	1253	08°29'49.5"	039°34'22.5"	60	72.5	2	NI
5	Jeju	HD	BR	1270	08°29'32.1"	039°35'21.8"	90	95.2	4	2018
6	Jeju	HD	BR	1268	08°29'34.7"	039°35'19.9"	70	75	4	2018
7	Adama	MI	BR	1477	08°24'10.8"	039°21'42.8"	51	10	2	2017
8	Lumie	Koka	BR	1598	08°27'19.7"	039°01'39.7"	60	93	2	2017
9	Lumie	Koka	BR	1601	08°27'14.4"	039°01'33.8"	45	40.8	2.33	NI
10	Lumie	Koka	BR	1595	08°27'08.9"	039°01'29.4"	60	72.7	2.75	2016
11	Lumie	Koka	BR	1591	08°27'01.9"	039°01'26.7"	70	94.7	3	2017
12	Lumie	Koka	BR	1607	08°21'51.2"	038°59'51.2"	70	36.4	2.33	2017
13	Lumie	Koka	BR	1598	08°21'36.0"	038°59'55.8"	90	46.7	2.33	2017
14	Bora	Kenteri	BR	1604	08°20'33.5"	038°59'14.6"	67	28.37	2.33	NI
15	Bora	Kenteri	BR	1601	08°20'02.6"	038°58'56.1"	70	20.01	2	NI
16	Bora	Elen	BR	1654	08°20'05.4"	038°56'31.4"	35	32.47	2	NI
17	Bora	Elen	BR	1599	08°20'27.8"	038°56'22.6"	66	23.27	2.33	2016
18	Dugda	KA	BR	1627	08°06'47.5"	038°47'45.2"	30	20.75	2	NI
19	Dugda	KA	BR	1629	08°06'47.7"	038°47'36.9"	90	87.18	4.33	NI
20	Dugda	KA	BR	1651	08°06'43.5"	038°47'12.3"	90	89.14	4.67	2018
21	Dugda	Abono	BR	1666	08°04'30.7"	038°45'03.9"	33	33.7	2	NI
22	Dugda	EC	BR	1633	08°00'22.1"	038°43'54.9"	60	50.18	3	2018
23	Dugda	EC	BR	1626	08°00'10.6"	038°43'53.9"	90	41.67	3.33	2018
24	Dugda	Alemtena	BR	1608	08°19'4.6"	038°58'8.6"	90	22.42	2.33	2018
25	Adama	MI	BR	1542	08°24'36.7"	039°19'32.5"	70	82.5	3	NI
26	Adama	MI	Nafis	1534	08°24'23.6"	039°19'56.6"	80	82.5	3	NI

Where, Var: variety; Alt: altitude; DAT: days after transplanting (days); DI: disease incidence; DS: disease severity; HD: Horota Dore; MI: Melkassa; KA: Korki Adi; EC: Elka Chelemo; BR: Bombe red; NI: no idea and YFFO: year of farmers first observation.

**Table 2.** Characteristic features of surveyed onion fields in the Central Rift Valley, Ethiopia.

District	Altitude (m.a.s.l)	Temperature range (°C)	Relative Humidity range (%)	Cropping system <sup>a</sup>
Dugda	1608.5 - 1666	20.55 - 31.75	37.55 - 73.25	sole
Bora	1599 - 1654	28.90 - 38.90	33.10 - 60.25	sole
Lumie	1591 - 1607	26.70 - 34.40	28.95 - 46.30	sole
Adama	1477 - 1542	24.10 - 29.85	31.50 - 58.30	sole
Jeju	1253 - 1270	30.10 - 31.35	33.20 - 33.50	sole

<sup>a</sup>Only dominant cropping systems are indicated. A.Chu.-Ada Chukallaa; AJTSC-African Juice Tibila Share Company.

belonged to small, private farmers. Each field was visited once. Sampled plants were randomly selected by using a quadrant (0.5 × 0.5 m) dropped on specified number of equally spaced paces following an inverted “X” pattern. Having made the pre-set number of quadrants (according to the size of the field), 3 - 5 plants/quadrant showing the suspected disease symptoms, as shown in **Figure 1**, were sampled.

### 2.3. Diseases Assessment

Disease incidence was assessed by counting the total number of plants having SLB symptoms divided by the total plant population within the quadrant and expressed in percentage. Disease severity was determined by a 0 - 5 visual scale (where: 0: no disease; 1: minute pinhead size spots, 1 - 10% diseased leaf area; 2: 11 - 20% diseased; 3: 21 - 40% diseased; 4: breaking of leaves from center, 41 - 75% diseased leaf area; 5: coalescing lesions with >75% diseased area), Sharma [23].

### 2.4. Isolation and Pathogenicity Test of the Pathogen

Small pieces of tissue (5 - 10 cm) containing lesions were incubated in moist chambers under light for 4 days at 25°C ± 2°C to induce sporulation and confirm colonization by *S. vesicarium*. After 4 days of incubation, conidial and mycelial masses were purified on to potato dextrose agar (PDA) and incubated for 10 - 16 days at 25°C ± 2°C. Cultural, morphological and pathogenicity test were used for identification.

Virulence test of the isolates recovered was carried out on Nafis onion variety. Pure culture of 14-days-old *S. vesicarium* was flooded with sterile tap water and, after gently rubbing with a rubber spatula, the resulting suspensions were filtered through sterile nylon gauze. Concentrations of the conidial suspensions were determined with a haemocytometer, adjusted to 3 × 10<sup>4</sup> conidia mL<sup>-1</sup>, Köhl *et al.* [7] with sterile tap water to inoculate 45 days old seedling in the greenhouse. The inoculated onion plants were kept up to 45 days in pots in the greenhouse at 27°C average temperature and 75% average relative humidity.



**Figure 1.** Onion field affected by stemphylium leaf blight: (a) early leaf death and (b) reduced bulb size.

### 3. Results and Discussion

#### 3.1. Symptoms

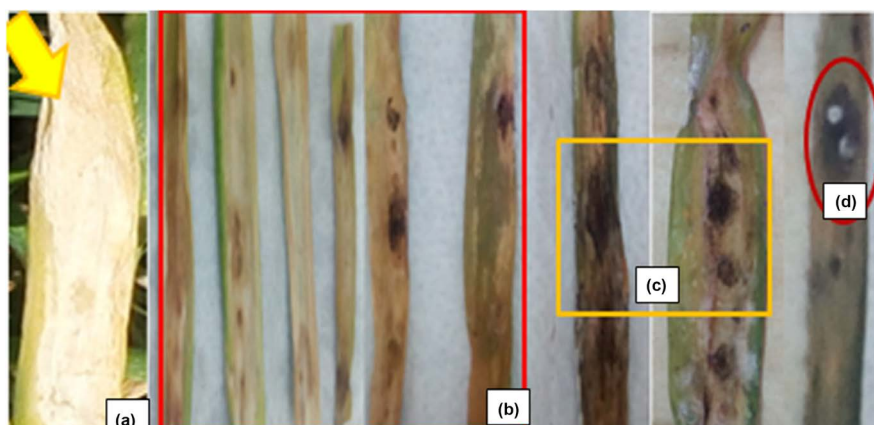
During the survey, the overall look of onion fields affected by the disease is depicted in **Figure 1**. Early senescence of leaves and flower stalks was observed, especially, when the severity of the disease was high (**Figure 1(a)**). Besides, reduced bulb size (**Figure 1(b)**) and associated yield loss was observed whenever matured onion fields were encountered during the survey. This was further confirmed from the communication made with producers during the assessment. Due to this massive appearance of the disease symptoms and maximum production loss, we were even told that farmers in the area call it as “*Yeshinkurt Ebola*”, meaning “Ebola of onion”. Specific disease symptoms of small to advanced tan to brown, water-soaked lesions on leaves and onion stalks, advancing to dark-brown to black sporulation and whitish fruiting structure were common (**Figures 2(a)-(d)**). These symptoms were more obvious on older plants. Overall, the symptoms observed during the survey are similar with symptoms description of SLB by Basallote-Ureba *et al.* [24].

#### 3.2. Disease Incidence and Severity

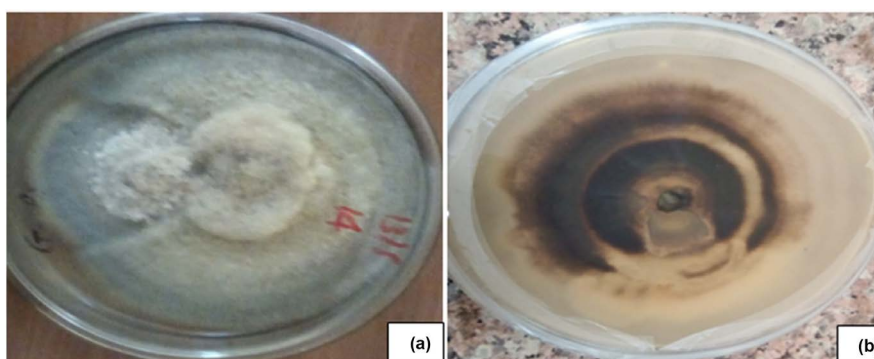
Severity and incidence of SLB in the surveyed area are summarized in **Table 1**. Accordingly, the highest (95.2%) and the lowest (10%) mean disease incidence was recorded at Jeju and Adama districts, respectively; while the maximum disease severity (4.67) was observed at Dugda. In general, SLB was prevalent in all districts assessed with various range of incidence and severity. According to the farmers, in areas such as Koka, the SLB disease was started to be observed since 2016, which might have been misdiagnosed as purple blotch disease.

#### 3.3. Identification of the Pathogen from Diseased Leaf Samples

Cultural, morphological and pathogenic characteristics of the isolates from symptomatic onion leaf specimen revealed the presence of *S. vesicarium*.



**Figure 2.** Symptoms of various stages observed during survey: (a) tan spots (lesions); (b) advanced brown spots; (c) brown lesions advanced to black sporulation; (d) advanced black sporulation tuning to whitish fruiting structure, mycelia.



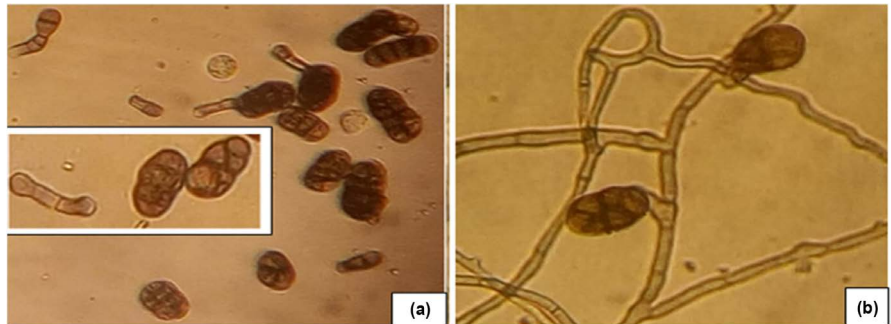
**Figure 3.** Cultural characteristics (a: front and b: back view) of 13-days-old *S. vesicarium* on PDA media.

### 3.4. Cultural and Morphological Characteristics

The in-face colony color was dirty white to light grey (**Figure 3(a)**). The reverse colony colors were deep brown, brown and light brown with circular white and brown alternate band growth pattern (**Figure 3(b)**). This dirty white and brown alternate growth and sporulation have found a distinct feature to *S. vesicarium* colony growth on PDA media. This result is equivalent to pathogen descriptions of Hosna *et al.* [25]. The conidia were olive-brown, oval to ovoid, oblong and ovoid to oblong, are borne on conidiophores that are pale to brown with dark edges and bands (**Figure 4**). This is in line with the *S. vesicarium* previous description, Simmon [26]; Basallote-Ureba *et al.* [24] and Woudenberg *et al.* [15].

### 3.5. Pathogenicity of *Stemphylium vesicarium*

Pathogenicity test was conducted to confirm whether the pathogen is the primary cause of the disease observed on onion fields during the survey. Periodical assessment (*i.e.* every week after 1<sup>st</sup> symptom observation) resulted in small white tan spots starting from 14 days of post-inoculation (**Figure 5**). The symptoms



**Figure 4.** Conidia (a) and Conidiophores (b) of 13-days-old *S. vesicarium* on PDA under 40× compound microscopy.



**Figure 5.** Symptom expression of *S. vesicarium* during pathogenicity test; (a) non-inoculated onion (b) inoculated.

have been intensive and extended to all inoculated plants with in a pot and were as similar to the symptoms observed during the survey. Moreover, successful re-isolation of the pathogen from artificially inoculated symptomatic leaf tissue affirms that the pathogen was pathogenic to onion and that was really responsible to massive production loss observed during the survey, which the onion farmers in the CRV area call it “Ebola of onion”.

#### 4. Conclusion

Based on the literature, this is the first report of the occurrence of stemphylium leaf blight (*S. vesicarium*) on onion in Ethiopia. Complementary work to map its distribution and intensity in the country, determining other putative hosts and the best management options will be relevant to manage the disease.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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