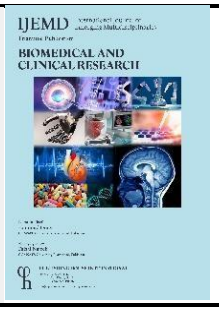
IJEMD-BMCR, (3) 1 (2025) https://doi.org/ 10.54938/ijemdbmcr.2025.03.1.417

International Journal of Emerging Multidisciplinaries:
Biomedical and Clinical Research

Research Paper
Journal Homepage: www.ojs.ijemd.com ISSN (print): 2957-8620 ISSN (online): 2960-0731



First Report of Powdery Mildew Diseases on Field Pea (P. Sativum Linn) in Northern Guinea Savannah of Nigeria.

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Abstract

Pisum sativum (field pea) belongs to the family Fabaceae. It is one of the six major pulse crops cultivated globally and is second highest yielding legume. A survey was conducted in Northern guinea savanna of Nigeria to identify the causative fungi of powdery mildew diseases on field pea. Temporay slides were prepared in the field by placing a clear tape on the infected part of the plant sticky side down and the tape was placed on a drop of distilled water/lactophenol cotton blue on a slide then observed under the microscope. The size and shape of the conidia and conidiophore were recorded. Samples were then sent to the Commonwealth Agricultural Bureaux International (CABI), UK for confirmation of the identification for the fungi inducing the diseases. Results obtained from CABI showed that four different species of *Erysiphe* i.e. *E. pisi, E. brunneopunctata. E. cruciferarum* and *E. cichoracearum* were responsible for the powdery mildew diseases observed on *P. sativum* in the study areas. As an exotic plant, the fungi might have come along with the seeds into the country, as *Erysiphe* spp. is seed borne or other reservoir hosts of the fungi might have occurred in the wild. To the best of our knowledge, this is the first report of the four species of *Erysiphe* inducing powdery mildew diseases of field pea in Nigeria.

Keywords: *Pisum sativum*, Powdery Mildew, *Erysiphe* spp.

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INTRODUCTION

Pisum sativum (field pea) belongs to the family Fabaceae. It is one of the six major pulse crops cultivated in different parts of the world. The crop is consumed both as fresh vegetable as well as in a dried form. Field pea can substantially substitute the protein sources of people on a starchy diet, particularly in a many places where most of the diets are carbohydrates based. This is why the introduction of the crop has received wide acceptance in Northern Nigeria [17]. The crop is grown mainly as cash crop in Nigeria, for example in 2017 a bag was sold at ₦ 250,000: 00 ($ 694.44) per 100Kg and upto today the price is still appreciating [13]. The plant was found to be cultivated under irrigation in few states in the Northern guinea savanna of Nigeria especially Kaduna, Katsina and Plateau States.

A number of factors militate against field pea production. [20] highlighted both abiotic and biotic factors. The abiotic factors include; frost, drought and excessive heat while the biotic factors include; pests and diseases. Fungi are among the biotic agents causing diseases of the crop. Pea powdery mildew caused by an obligate organism is one of the most important fungal disease in several cultivated crops with world wide distribution [5]. The disease have been reported to be caused by several species of *Erysiphe* [10].

Powdery mildew and wilt diseases are caused by members of Ascomycota, which causes 25 – 80 % yield losses [4]. Early infections completely destroy the crop while occurrence of the disease at podding stage reduced the grain yield [10]. [14] reported high incidence of powdery mildew diseases which is favored by both maximum and minimum temperatures in Sabon gari and Sabuwa local government of Nigeria in 2016/2017 and 2018/2019 growing seasons. The aim of this study is to identify the causative fungi of powdery mildew diseases of field pea in Northern guinea savanna of Nigeria.

MATERIALS AND METHODS

Sample Collection

Samples of field pea plant with powdery mildew symptoms were collected from November to April for two seasons (2016/2017 and 2017/2018) from different farms in irrigated fields of Northern guinea savanna of Nigeria (Katanga and Shika dam in Kaduna state and Sabuwa in Katsina state). The leaves, stem, petiole, stipules, tendrils and pods were examined for symptoms of powdery mildew. Razor blade was used to cut infected parts of the plant and dried in between papers in a plant press. Geographic Positioning System (GPS) was used to take the coordinates of each sampling site.

Diseases incidence for in each farm was calculated as;

$$\text{Diseases Incidence} = \frac{\text{Number of Disease Plants}}{\text{Total Number of plants acessed}} \times 100 \quad [9].$$

Identification of Powdery Mildew Fungi from *P. sativum* in Irrigated Fields of Northern Guinea Savanna of Nigeria

Temporay slides were prepared in the field by piece of clear tape. it was hold on one end of the tape and lightly smoothed the rest of the tape sticky side down over the infected plant part the tape was placed sticky down on a drop of distilled water/ lactophenol cotton blue on the microscopic slide. The slides were observed under the microscope. The length and width of 100 conidia and conidiophore were measured in micro meters as described by [12] and [3]

Molecular Identification of Powdery mildew Fungi

Powdery mildew samples were sent to Commonwealth Agricultural Bureaux International (CABI), UK for confirmation of identification. The samples were processed following DNA extraction, Polymerase Chain Reaction (PCR) was employed to amplify copies of the DNA. The PCR products were then sequenced (CABI, 2019).

RESULTS

Symptoms and Morphological characteristics of Powdery mildew Fungi Isolated from *P. sativum* in irrigated fields in Northern Guinea Savanna, Nigeria.

Three different types of powdery mildew symptoms were observed on different parts of field pea plant.

Whitish powdery patches of mycelia

Whitish powdery patches of mycelia and conidia which occur on both abaxial and adaxial leaf surface, stem and tendrils, it then coalesced as infection becomes severe. The samples were found to be mixed infection containing two different fungi, the first one under the light microscope were ellipsoidal or ovoid hyaline conidia which are produced singly on aseptate conidiophores. The size of conidia ranged from 28 x 12 µm to 41 x 19 µm length/width ratios. Length of conidiophore ranged from 70 to 84 µm, width 6 to 8.4 µm, identified as *Oidium* sp. (anarmorph), *Erysiphe pisi* (teliomorph) as shown in Table 1 and Plate 1. Similarly, the second fungi observed under the microscope, have ovoid hyaline conidia produced singly on conidiophore, the fungi was identified as *Oidium* sp. (anamorph) and *E. brunneopunctata* was identified as the teliomorph stage as indicated on Table 1 and Plate 2.

Whitish powdery dispersed mycelia on leaves and pods

The whitish powdery dispersed mycelia which later turns brownish as infection becomes severe on aerial parts leads to observation of cylindrical to ellipsoidal conidia borne singly on conidiophore with sizes ranging from 36 x 14 µm to 43 x 17 µm length/width ratio. The fungi was identified to be *Oidium* sp. (anamorph) and molecular analysis revealed *Erysiphe cruciferarum* as teliomorph as shown in Table 1 and Plate 3.

Whitish powdery mycelia on leaves

Whitish powdery mycelia on leaves showed ellipsoidal or ovoid conidia with varying sizes of 26 x 16 µm to 34 x 19 µm length/width ratio *Oidium* sp. (anamorph) and molecular identification *E. cichoracearum* (teliomorph) was identified from the samples as shown in Table 1 and Plate 4

Confirmation of identification by CABI showed results of four different species of *Erysiphe*. Sample SA1 was identified as *E. pisi* with 100 % to *Erysiphe* sp. with accession number JX162391, FIESC strain CBS 131787. Sample SA1b was identified as *E. brunneopunctata.* Sample SA3 was identified as *E. cruciferarum* ITS sequence showed top matches at 100 % to *E. cruciferarum* (EF551461) Sample SA6 was identified as *E. cichoracearum* the ITS sequence showed >99 % identify to sequences of *E. cichoracearum* (KP131956).

Distribution and Incidence of Powdery Mildew diseases in Relation to Sampling Locations

Katanga is located at longitude 11º10'13" and latitude 7°46'39". Shika dam is located at 11.4ºN and longitude 7.75ºE. Sabuwa irrigated fields were located at 11°10'13"N and latitude 7°46'39"E in Katsina state. During field survey, it was found that farmers grow three different varieties of field pea differentiated based on the color of the seeds (green seed, gray seed and white seed varieties) in all the sampling locations (Katanga, Shika dam and Sabuwa). All the four fungal isolates (*E. pisi, E. brunneopunctata, E. cruciferarum* and *E. cichoracearum)* were isolated from the green seed, gray seed and white seed varieties of field pea in Katanga and Shika dam Kaduna State. However, only *E. pisi* and *E brunneopunctata* were isolated from green seed and gray seed in Sabuwa in Katsina state as shown in Table 2.

The incidence of powdery mildew disease was higher in Katanga irrigated fields on both the white seeded variety (100 %) and green seeded variety (50%) followed by Shika dam with (90, 40%). While Sabuwa irrigated fields had the least percentage incidence of powdery mildew diseases where it was absent on the white seeded variety and only 30 % incidence was observed on the green as shown in Figure 1.

Table 1: Morphological Identification of Powdery Mildew Fungi Isolates in *P. sativum* in Irrigated Fields in Northern guinea Savanna

SYMPTOMS TYPE	MICROSCOPIC CHARACTER	CONIDIA SIZE (µm)	NAME OF FUNGI
Whitish powdery mycelia that occur in patches on both upper and lower leaf surface which coalesced as infection become severe.	Conidia ellipsoidal or ovoid hyaline produced singly on aseptate conidiophore which arises from epiphytic hyphae.	Length ranges from 28 µm to 41µm. Width ranges from 12µm to 19µm. length of conidiophore ranges from 70 to 84µm. width ranges from 6 to 8.4µm.	Anamorph: *Oidium* sp. Teliomorph: *Erysiphe pisi*
	Conidia ovoid hyaline produced singly on aseptate conidiophore which arises from epiphytic hyphae.	Length = 25 to 39 µm. Width = 11to 16 µm.	Anamorph: *Oidium* sp. Teliomorph: *Erysiphe brunneopunctata*
Whitish powdery dispersed mycelia which turns brownish as infection becomes severe on leaves, stems, making pods to crack.	Conidia ellipsoidal to cylindrical hyaline produced singly on aseptate conidiophore which arises from epiphytic hyphae.	Length = 36 to 43 µm. Width = 14 to 17µm.	Anamorph: *Oidium* sp. Teliomorph: *Erysiphe cruciferarum*
Whitish powdery dispersed mycelia which turns brownish as infection becomes severe on leaves, stems, making pods to crack.	Conidia ellipsoidal or ovoid hyaline produced singly on aseptate conidiophore which arises from epiphytic hyphae.	Length = 26 to 34 µm. Width = 16 to 19µm.	Anamorph: *Oidium* sp. Teliomorph: *Erysiphe cichoracearum*

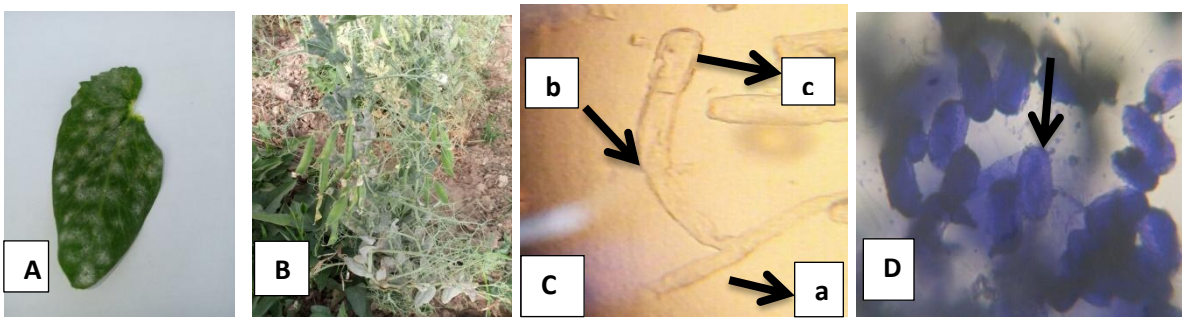

Plate 1:A and B= Symptoms of powdery mildew disease cause by *E. pisi* on *P. sativum* C= Conidiophore bearing conidia. (a= hyphae, b= conidiophore, c= conidia) of *E. pisi*. Mounted in Distilled water D= conidia of *E. pisi*. Stain; Lactophenol cotton Blue. Mg= x40.

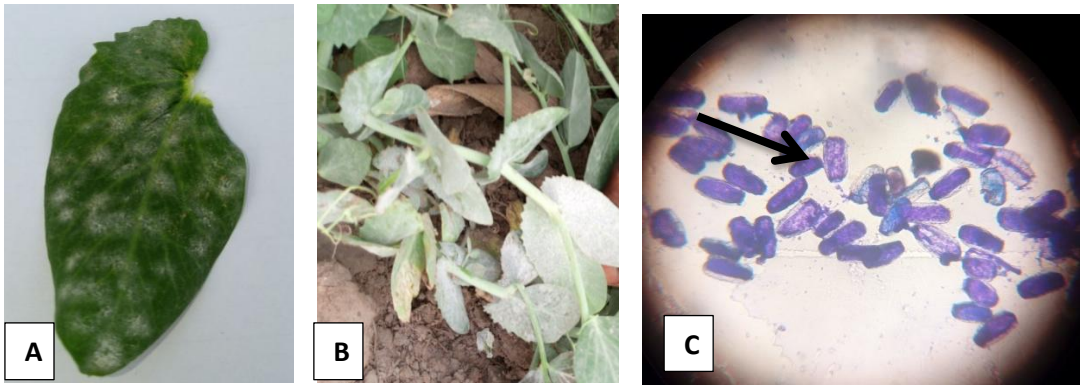

Plate 2:A and B= Symptoms of powdery mildew disease cause by *E. brunneopunctata* on *P. sativum.* C= conidia of *E. brunneopuctata*. Stain; Lactophenol cotton Blue. Mg= x40.

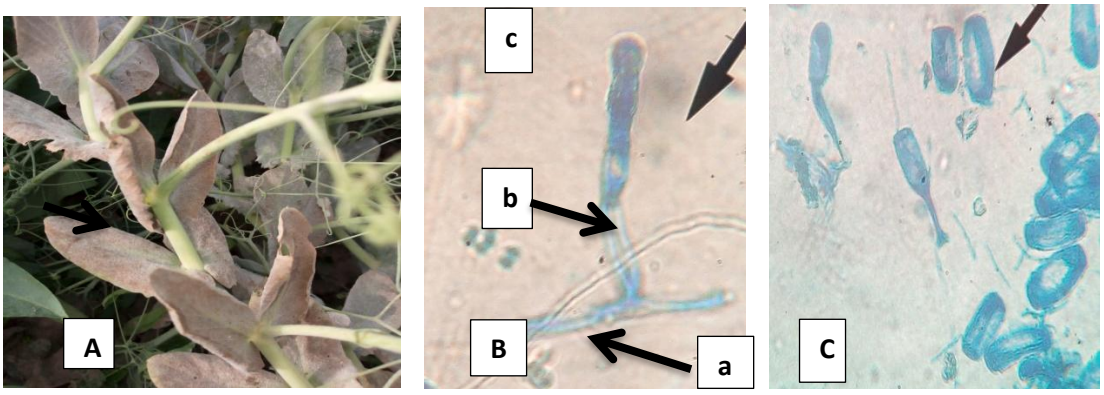

Plate 3:A = Symptoms of powdery mildew disease cause by *E. cruciferarum* on *P. sativum* B= Conidiophore bearing conidia. (a= hyphae, b= conidiophore, c= conidia) of *E. cruciferarum* C= conidia of *E. cruciferarum*. Stain; Lactophenol cotton Blue. Mg= x40.

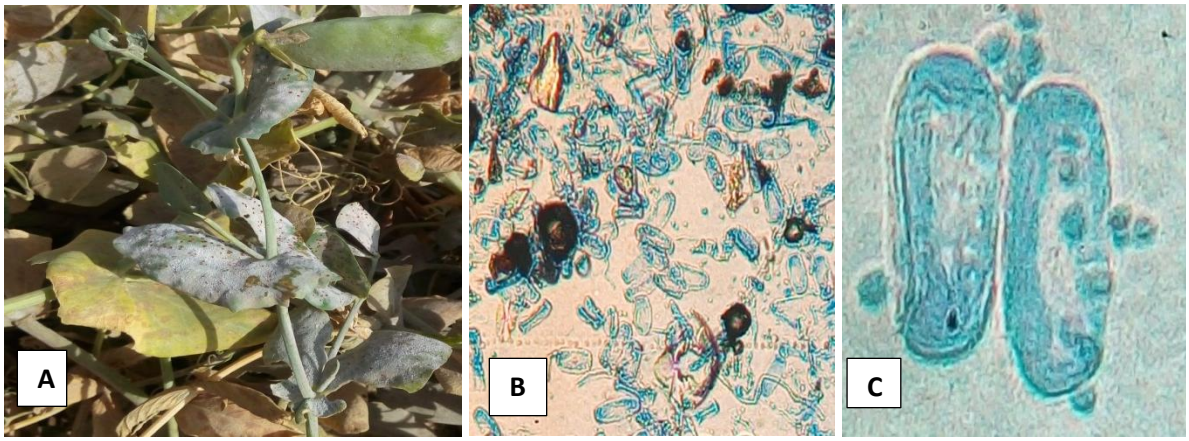

Plate 4:A Symptoms of powdery mildew disease cause by *E. cichoracearum* on *P. sativum*
B= conidia of *E. cichoracearum.* Mg= x10
C= conidia of *E. cichoracearum*. Stain; Lactophenol cotton Blue. Mg= x40.

Table 2: Distribution of *Erysiphe* species on Irrigated Field Pea in Northern Guinea Savanna of Nigeria.

Locations	*E. pisi*	*E. brunneopunctata*	*E. cruciferarum*	*E. cichoracearum*
Katanga	+	+	+	+
Shika dam	+	+	+	+
Sabuwa	+	+	-	-

Key; + = Present - = Absent

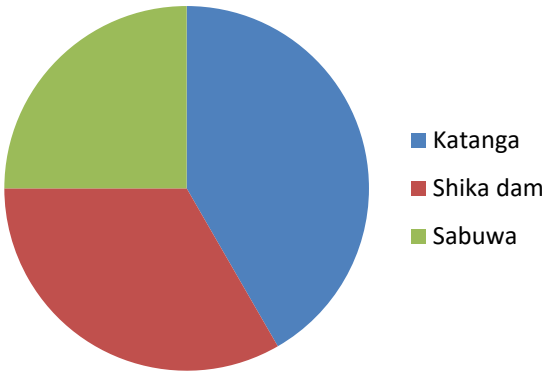

Fig 1: Pie chart showing Incidence of Powdery mildew diseases of Field pea Grown under Irrigation in northern Guinea Savanna of Nigeria

DISCUSSION

The symptoms of powdery mildew observed in this study were similar to the reports of [21], [11], [10]. Morphological characteristics of the pathogen *Oidium* sp. observed in these findings is in line with the results of [12], [23]. However, there are variations in the size and morphology of conidia and conidiophore of the different *Oidium* spp. observed. The size of conidia for *E. cruciferarum* and *E. cichoracearum* were similar to the report of [1], [15].

Samples containing *E. pisi* often referred to as *E. polygoni* were found to be mixed with *E. brunneopunctata* making it difficult to differentiate the symptoms. However, based on the morphology of the conidia it was observed that *E. pisi* was dominant over *E. brunneopunctata.* This suggests that the field pea plant might be more susceptible to *E. pisi* than *E. brunneopunctata.* There is no report of these two fungal pathogens occurring together. However, [18] reported other *Erysiphe* spp. to have occurred on same host plant (oak). [24], [19] have also reported *E. pisi* on field pea in many parts of the world Africa inclusive. *Erysiphe brunneopunctata* has been reported to cause powdery mildew on different hosts plants not field pea [7].

Powdery mildew induced by *Erysiphe cruciferarum* on field pea was found in Katanga and Shika dam irrigated fields. The pathogen majorly causes powdery mildew of crucifers especially cabbage, broccoli [2], [25], [15] in different parts of the world. *Erysiphe cichoracearum* was isolated from samples obtained from irrigated fields of Katanga and Shika Dam. The pathogen had been reported on weeds in Zaria for example *Bidens binnata* [8], [1]. This may suggest transmission of the pathogen from these weeds to *P. sativum* as the above weeds were found growing at the ages of farms in the study area.

The powdery mildew symptoms were mostly observed on the newly imported variety (white seeded variety) this suggest that the fungal pathogens might have come along with the seeds as it has been also reported as seed- borne. [10], [22], [16], [26] reported the occurrence of *E. pisi* on other plant hosts which include; cabbage; onions, guava, *Pisum arvense,* and pigeon pea. During field survey, it was observed that most of the fields the pea was intercropped, in rotation or grown adjacent these crops this may suggest that the plants may harbor the pathogen which was transmitted to the field pea plant although no visible symptoms was observed on them during this study. Also the powdery mildew fungi might have been transmitted through surface run- off irrigation or wind from other crucifers to the field pea plants. Also poor sanitation was observed as crop residues were not destroyed properly which might have served as carry over inoculum to the next growing season.

The difference in the incidence of powdery mildew diseases on white seeded variety and other varieties (gray and green seeded varieties) across the sampling locations may be attributed to the presence of water reservoir near Katanga and Shika dam which might have enhanced the development of disease compared to Sabuwa where farmers use bore-hole as source of irrigation. To the best of our knowledge this is the first report of *E. pisi, E. brunneopunctata, E. cruciferarum* and *E. cichoracearum* inducing powdery mildew in field pea in Nigeria.

CONCLUSION

Powdery mildew diseases of irrigated field pea in Northern guinea savanna of Nigeria are induced by *E. pisi, E. brunneopunctata, E. cruciferarum* and *E. cichoracearum.*

ACKNOWLEGEMENT

Special appreciation goes to Commonwealth Agricultural Bureaux International (CABI), UK, Mal. Ibrahim Bello Department of Zoology and Mal. Isa Abdul Jibrin Department of Crop Protection Ahmadu Bello University Zaria, Nigeria for Technical assistance.

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