

# Survey for pink Root Rot of Onion (*Pyrenochaeta terrestris*) and its identification In Gezira State

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**Abstract:** This survey was conducted in Gezira State to detect the pink root rot disease of onion, caused by *Pyrenochaeta terrestris* in Gezira State. The study evolved the isolation, identification of the causal agent of determination of the level of the disease incidence. Three locations within Gezira State were selected namely the vicinity of Almusallamih Tayiba, Wad Al ataya and Hamdalnil and located at North, central and south of the State respectively. The results showed that the local variety was found to be highly susceptible to the disease than the exported of the hybrid ones. The highest disease incidence was recorded in Hamdalnil (16.8%) while the lowest disease incidence was recorded at Wad Al ataya(9.23%). Koch's postulates were performed to prove that the fungus isolated *Pyrenochaeta terrestris* was the causal agent of the pink root rot on onion plants. The successful isolation of the fungus and the verification of its Pathogenicity test revealed the way for further epidemiological studies of the said disease.

**Keywords:** Onion, pink root rot, *pyrenochata terrestris*, control

## Introduction :

Onion (*Allium cepa*.L.) is a biennial vegetable plant which plays an important role in human life, both as food or as a cash crop. Onion contains essential nutrient elements. It contains intermediate protein and considered to be rich in calcium and riboflavin (Thompson and Kelly, 1957). In Sudan, onions ranks first, followed by tomato in terms of acreages of land devoted for the crop. It is grown all over the country. It is mainly concentrated in the central and northern states in addition to Darfur and Kassala. It occupies about 25% of the area under vegetable production (Ahmed and Mohamed, 1997).The Pink root disease organism has passed through several names. First, Hansen (1926) declared that the causal organism of the disease was *Phoma sp.* and he named it *Phoma terrestris*. Other workers depended on the presence of setose on the pycnidium for giving the name *P. terrestris* (Hansen) nov.comb (Gorenz *et al.*, 1948). Lastly, the name was conventional as *Pyrenochaeta terrestris* by (Kodama *et al.*, 1976). The fungus forms an aerial mycelium which can be described as septate, guttulate, compact, delicate, velvety grey in color and released pink to purple pigmentation in the culture media. The pycnidium shape is globose to subglobose, immersed then erumpent, ostiolate, papillate and dark brown to carbonaceous. Setose might occur singly, but frequently in clusters are dark brown and abundant around the ostiole. Conidia are continuous, hyaline, biguttulate, escaping as a gelatinous mass through ruptures. The hyphal cells first appear as swelling and then divide to give rise to conglomerate masses of dark

thick walled bodies, somewhat resembling chlamydospores, and known as microsclerotia or resting bodies of the fungus *P. terrestris* (Elamein, 1999). On the other hand, some workers mentioned a number of *P. terrestris* isolates from different locations which vary in their growth characteristics and virulence on onion plant. From Sudan, Elamein (1999) mentioned several isolates, namely Hudeiba, Gezira, Masalamyiah, Islanj, Kassala, Wad albasal and Hilalyiah. Al-saggaf (1997) also reported one isolate in Yemen which was designated Hudramout isolate. So the objectives of this study are to: (a) Isolate and identify the causal organism of pink root disease of onion in Gezira scheme, (b) get acquainted with disease incidence in different areas surveyed.

#### **Material and Methods:**

A field survey was conducted to determine the extent of pink root infestation in Gezira onion fields. During November 2017 in Gezira state when the crop was at physiological maturity. The survey was carried out from distractive areas viz.(Almuslimih tayiba) section that is located in northern part of Gezira scheme and Hamdالنيل inspection of middle section and the inspection of Wad Alataya south section. A survey was carried out in the area represented by about four fadan for each location. In each location five samples of the four directions were taken and the central area of the field was covered in 25square maters of each sample (figure1). The data collected were expressed a percentage of disease plant out of the total of plant inspected as fallow

Disease incidence:  $\frac{\text{No. of infected plants}}{\text{Total No. of plant assessed}} \times 100$

Total No. of plant assessed

#### **Isolation of causal organism:**

Isolation of causal organism infected onion plants with prominent pink-root disease symptoms were selected and their roots were first washed in running tap water for 20 min, then cut into small pieces (0.5 – 1cm), soaked in 0.5% sodium hypochlorite (10% Chlorox) for surface sterilization, rewashed in sterilized water and dried between two pieces of sterilized filter paper. Three root pieces were inoculated equidistantly, in each Petri dish containing Watson's medium (W.M) with sterilized wheat straw. The medium was prepared by dissolving 3g Sodium nitrate + 1g magnesium sulfate + 20g agar in

1000 ml distilled water, autoclaved at 121°C for 20 minutes and then tetracycline hydrochloride was added at the rate of 50 mg/l when the medium temperature came down to 60°C. The inoculated plates were incubated at 28°C for 4 to 7 days (Al-saggaf, 1997; Elamein, 1999; Adam, 2003). To obtain pure culture, subculture were made on potato Dextrose Agar medium (PDA) from growing hyphal tips showing pink coloration on wheat straw following Al-saggaf's procedure. The subcultures were incubated at 28°C for 7 days. Stock cultures were kept in slant in McCartney bottles by placing small pieces of the hyphae tips taken from the pure culture. They were incubated for two weeks at 28°C and then stored at 5°C to be used for further studies (Elamein, 1999). The samples brought for laboratory investigation were kept in paper bags and in polythene bags and stored in refrigerators for further investigation.

#### **Isolation from soil:**

Soil samples were taken randomly from infected plant field in plastic bags, after the soil was mixed thoroughly, one gram was taken and added to 100 ml sterilized distilled water. Five ml of this suspension were taken by 25 micro pipettes and added to other 100 ml of S.D.W. in test tubes. Three samples were taken for the purpose of the study. To each of the four Petri dishes containing PDA, one ml suspension taken from the last dilution was added. The Petri dishes were incubated at  $\pm 28^{\circ}\text{C}$  and examined under the microscope for fungal growth.

#### **Isolation from pink root rot infected sample**

Roots of onions with typical pink root symptoms, were collected from plants grown on clay soil in Al Gezira state for different inspections, and received to lab in the college of agricultural study of Sudan university of science and technology, the roots were washed thoroughly under running tap water and plotted dry with a paper towel. To obtain stellar pieces of these roots, the peel of each root was removed, the root cut in to 5-mm long pieces dipped in to chlorox (0.5% sodiumhypochlorite) for 5-10 second and plated on filter paper towel dry, then a pieces were Plated on water agar (W.A) in Petri dishes (4-5) per plate and sealed with Para film and incubated under  $24^{\circ}\text{C}$  temperature. After four days, colonies grown on as the *Fusarium spp*, these colonies placed on other medium PDA, Watson, and wheat straw) and determined the presence of pink color on culture, confirmed to *P. terrestris* as the infected roots.

#### **Identification of the causal fungus:**

To identify the causal fungus, the morphological as well as cultural characteristics of the isolated fungus were recorded in W.M+ wheat straw incubated at 28°C for 14 days.

Verification was conducted through charts, descriptions of CMI for pathogenic fungi and bacteria (1970) and books of Demataceous Hyphomycetes Ellis (1971) and genus *Fusarium* by Booth (1971).

#### **Pathogenicity Test:**

Soil infestation was made by mixing 2 kg of sterilized soil with 100 g of fungus inoculums (Al-saggaf, 1997). Ten pots were used. The soil of five pots artificially, infested while that of the other five pots was not infested to serve as control. All pots were watered for two days prior to seed sowing to activate the fungus. After 5-8 weeks the seedlings were up-rooted and examined for symptoms development.

#### **Experimental design**

The experiment was arranged in a Complete Randomized block Design.

#### **Statistical analyses**

The obtained data was statistically analyzed according to analysis of variance (ANOVA) Duncan's Multiple Range Test (DMRT) was used for means separation using Mstat-C statistical package (MSTAT-C .1991).

#### **Results and Discussion:**

This study was carried out in Al- Gezira scheme, since it has not been previously surveyed only surveyed area is Zalingi region  
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in west Darfur. However, all studies were based on comparisons between species and their degree of resistance to disease. During the present investigation a field survey was conducted to gather data on the severity of pink root rot of onion from growing distractive area on Gezira State. Survey was demonstrated that the majority of areas were affected by the fungus *Pyrenochaeta terrestris* such as (Al muslimih tayiba) section that is located in northern part of Gezira scheme and Hamdalnil inspection of middle section and the inspection of Wad Alataya south section. A survey was carried out in the area represented by about four fadan for each location. The result in Table 1 Figure 1 showed that there was a difference in disease incidence of pink rot disease in the different scheme. It was found that the rate of infection in the northern section of the almuslimih bureau of tayiba (11.07) and in the central inspection Hamdalnil (16.8) in the southern section of inspection of Wad al Ataya (9.23) . It is clear from these percentages that Hamdalnil inspection is the most affected area that is because the farmer in this area used to grow the local variety 'sagi' which recorded in our results the highest incidence, on the other hand farmer were ont aware of the disease and its importance. However, different local names of the disease are used in different areas of study. i.e.(Alhanani and Alhimor). All these facts above lead to the high incidence in this area (Hamdalnil). The isolate confirmed as *Pyrenochaeta terrestris* on the wheat straw on PDA (figure2) (Watson1961). The isolate found in the present study matched the morphological description of *Pyrenochaeta terrestris* in culture by (Kinsey, 2002).(figure 3&4)

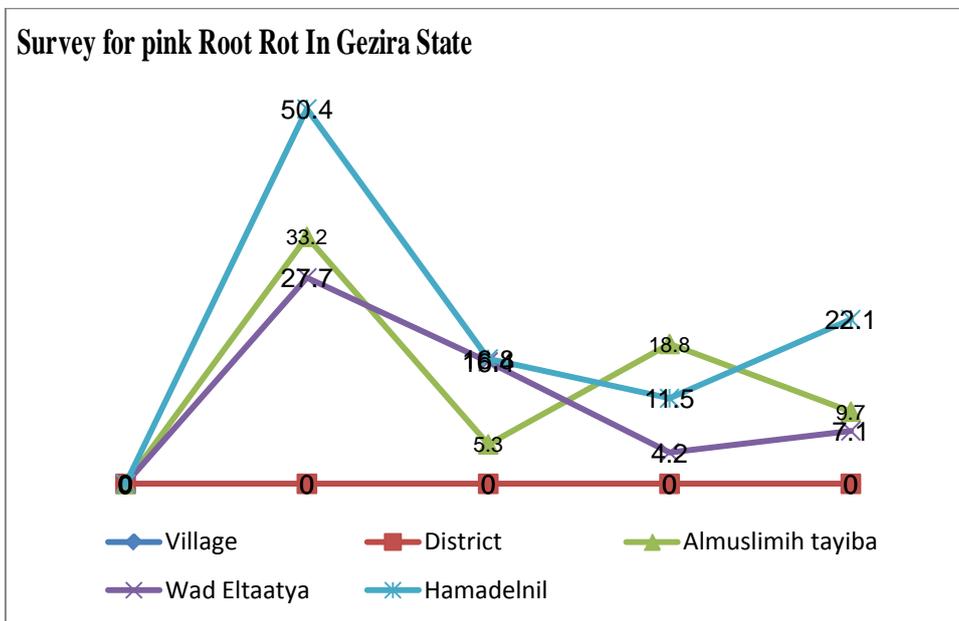


Figure2 Survey for pink Root Rot of Onion (*Pyrenochaeta terrestris*) In Gezira State



Figure 3. The survey site showed Onions with pink root in Gezira field



Figure 4. Onion roots with symptoms of pink root disease Fig. 3. fungal growth on the wheat straw on P.D.A.

### Pathogenicity Test:

In the Pathogenicity test, the roots of the inoculated seedlings showed typical pink root disease symptoms, water soaked areas, turned yellow and shriveled or turned purple and pink to purple color. It became semitransparent and could be easily peeled-off or severed. The leaves became dry and the tip withered. The re-isolated fungus gave similar cultural characteristics to the original culture isolate from the symptomatic (pink root-affected) onion seedlings. All Koch's rules were satisfied and the isolated fungus was verified as the causal pathogen of the pink root disease in onion plants under study (Kinsey, 2002).

### CONCLUSION:

This survey showed that onion roots can be colonized by various pathogenic fungi. However, the impact of these pathogenic fungi depends on their distribution (frequency) and infection levels (severity). It also confirmed the presence of *Fusarium spp.* Further studies are required to better understand onion root pathogen interactions and the overall impact of these root diseases on onion crop and ultimately yield. The successful isolation of this fungus and verification of its pathogenicity reveal the way for epidemiological study of this disease.

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