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Full Length Research Paper

Isolation and identification of a bacterial blotch organism from watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai)

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A study was carried out on the fruit rot of watermelon (*Citrullus lanatus* (Thunb.) Matsum. Nakai) in Ilorin, Nigeria between February and May, 2008. A large area planted to watermelon in Obbo-ille, a town near Ilorin, Kwara State was severely infected with both leaf and fruit blotches. Both diseases occurred together in all infected watermelon stands. Leaf and fruit samples were taken to the laboratory at the University of Ilorin, for investigation. A bacterial isolate identified as *Pseudomonas pseudoalcaligenes* Stanier (= *Acidovorax avenae*) was recovered from both infected leaf and fruit samples. Koch's postulates were performed on both samples using a suspension of the culture isolate. Symptoms were produced on artificially inoculated leaves and fruits and *P. pseudoalcaligenes* was also re-isolated. This organism was, therefore, identified as the causal organism of bacterial fruit and leaf blotch in watermelon in Ilorin, Kwara State of Nigeria.

Key words: Watermelon, fruit, leaf, blotch, *Pseudomonas* sp.

INTRODUCTION

In a hungry world with an ever-increasing population, it becomes imperative that efforts are made at reducing crop loss due to pathogen infestation. Much of the yield of watermelon fruits is lost to Bacterial Fruit Blotch (BFB), a yield-reducing disease. Watermelon (*Citrullus lanatus*) belongs to the family Cucurbitaceae. According to Dutta (1979), plants in this family are mostly used as vegetables, a few yield delicious summer fruits and a few are medicinal. Other members of this group include sweet gourd or muskmelon (*Cucurbita moschata*), pumpkin (*C. pepo*), *Cucumis* spp. and *Momordica* species. Watermelon originated in Africa and has been in cultivation for more than 4, 000 years in the drier parts of the continent and throughout India and parts of Asia (Cobley and Steele, 1976). It is used as a dessert fruit and a thirst quencher and in the very dry parts of Africa, it is relished by both man and his animals as a source of water.

Watermelon plant is a trailing hairy annual with rough, angular stems and dark green alternate leaves carried on fairly long petioles. Watermelons are monoecious and have pale yellow flowers which are smaller than those of pumpkins and squashes. The fruit is large and rounded or oblong with a hard, smooth rind often mottled light and dark green. The fruit contains a red, pink or yellowish-white flesh with numerous flattened, black, brown or white seeds. There is little food value in the flesh, with very little flavour and about 95% or more being made up of water (Cobley and Steele, 1976). The seeds, however, contain a considerable amount of oil.

Several diseases afflict watermelon (*C. lanatus*). Some of these include angular leaf spot (*Pseudomonas syringae* pv. *lachrymans)*, *b*acterial fruit blotch/seedling blight (*Acidovorax avenae* subsp. c*itrulli* = *Pseudomonas pseudoalcaligenes* subsp. *citrulli*), bacterial leaf spot *(Xanthomonas campestris* pv. c*ucurbitae*) *b*acterial rind necrosis (*Erwinia* spp.) and bacterial soft rot (*Erwinia carotovora* subsp. *carotovora*). Some fungal diseases include *Alternaria* leaf spot/blight (*Alternaria cucumerina*), anthracnose (stem, leaf and fruit) (*Colletotrichum orbiculare*), belly rot (*Rhizoctonia solani*), black root rot (*Thielaviopsis basicola)* and *Fusarium* fruit rot caused by *Fusarium equiseti* (Martyn et al., 1993; Roberts and Kucharek, 2006).

Bacterial fruit blotch and leaf blight seem to be the most

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Table 1. Rating scale for bacterial leaf blotch of watermelon (*Citrullus lanatus*).

Scale	Description	Inference
0	No symptoms on leaves	No Infection
1	1 - 25% leaf area covered with lesions	Mild Infection
2	26 - 50% leaf area covered with lesions	Moderate Infection
3	51 - 75% leaf area covered with lesions	Severe Infection
4	76% and above	Very Severe/Devastating

Five leaflets were selected at random among the 150 plant stands rated. Visual observation of the selected leaflets was carried out and the severity recorded. Results represent the mean rating.

important yield-reducing diseases of watermelon. According to Latin (2000), BFB was first reported in the Mariana Islands in 1988 and appeared in commercial watermelon fields in the United States in 1989. Although other cultivated cucurbits are susceptible to infection, significant losses have been reported only in watermelon crops (Latin, 2000; Anon, 2008). Symptoms of the disease include oily, water-soaked lesions on the top side of the developing fruit (Jett et al., 2002; Egel, 2007). According to Isakeit (1993), it is very likely that both weather conditions and cultivar in addition to other factors will affect symptom development from one location to another. Cool (60 F night and up to 77 F day) temperatures along with rain or continuous high humidity favour bacterial diseases in watermelon. Fruit blotch can occur in a field miles away from other cucurbits, suggesting that the spores that serve as inoculum are air-borne.

The aim of this study is to determine the causal agent(s) of watermelon foliar and fruit diseases in a farm at Obbo-ille in Kwara State of Nigeria as well as evaluate the importance of the disease in watermelon production.

MATERIALS AND METHODS

Survey and sample collection

A survey was carried out February – May, 2008 on a farm at Obbo-ille on the outskirts of Ilorin, Nigeria to determine the incidence and severity of fruit and leaf lesions observed in watermelon (*C. lanatus*). A rating scale (Table 1) was employed to assess the incidence and severity of the disease. The Disease Severity Index (DSI) was also calculated. Samples of infected leaves and fruits were collected and brought to the laboratory at the University of Ilorin for isolation and preliminary studies.

$$DSI = \frac{\text{Sum of individual ratings}}{\text{Number of plants assessed}} \times \frac{100}{\text{Max. disease category}}$$

Isolation and pathogenicity test

Isolations were made from leaves and fruits on Potato Dextrose Agar (PDA). Very small sections of infected leaves and fruits (1 mm diameter) were cut into two separate 10-ml beakers. The fruit and leaf sections were surface-sterilized separately in $NaOCl_3$ for 3 min and rinsed twice in sterile distilled water. The sections were blotted dry between sterile Whatman No. 1 filter paper and plated separately on sterile potato dextrose agar medium. Inoculated plates were incubated at room temperature (25 ± 2˚C) and colony development monitored. Emerging colonies were subcultured to obtain pure cultures. Microscope slides were prepared from the pure cultures and identification carried out using laboratory manuals.

Pathogenicity test was performed by smearing the surfaces of healthy watermelon leaves and fruits with a suspension of the isolate. Inoculated leaves and fruits were incubated in humid sterile plastic containers. Re-isolations were carried out from the artificially inoculated leaf and fruit samples.

RESULTS

Results of a field survey carried out on a large farm at Obbo-ille, Kwara State revealed that watermelon cultivated at that farm were badly infested with bacterial infections. The observed symptoms were those of both fruit and leaf blotch/blight and they were prevalent and severe. Out of 150 randomly selected plant stands that were sampled, 45% showed both leaf and fruit symptoms ranging from slight to severe. Both symptoms were present on most of the stands that showed symptoms. The initial symptoms consist of irregular contour of yellow lesions or streaks on the upper surface of infected *C. lanatus* leaves. The lesions later turned rusty-coloured and were usually near the edges of the leaves. Infected leaves appeared water-soaked on the abaxial (underside) surfaces. The Disease Severity Index (DSI) of the surveyed field was 48% that is, 2 on the rating scale, translating to moderate severity.

Fruit development was hampered by leaf symptoms resulting in the production of stunted fruits in many of the infected plants. Fruit infection (blotch) began as small dark-green, water-soaked lesions on any part of the fruit that is not directly in contact with the soil. The lesions enlarged rapidly and covered most of the fruit surface in 5 - 7 days depending on the fruit size and the weather conditions. As the lesions enlarged, cell necrosis occurred at the initial points of infection. In this study, it was observed that lesions enlarged faster on infected detached fruits placed on laboratory bench than on attached ones in the field. While complete damage may occur on attached fruits within 7 - 10 days, it took 6 - 8 days on detached fruits. It does appear that leaf lesions served as a source

of inoculum for fruit infection because in most cases, by the time symptoms were seen on the leaves, it was too late to prevent fruit infection.

Both foliar and fruit infections were found in this study to have very important effects on the production of watermelon. It was observed that leaf spots reduced the photosynthetic surface of the leaves available for food manufacture. This consequently resulted in reduction in fruit sizes in infected watermelon stands compared to fruits produced by healthy stands. It was also observed that in severe cases of fruit blotch, fruit rot occurred.

DISCUSSION

Results of this study showed that both fruit and leaf blotch in watermelon were caused by the bacterium *P. pseudoalcaligenes.* Investigations regarding the proof of pathogenicity confirmed the isolated bacterium to be responsible for the disease. The two diseases occurred together in the fields surveyed at Obbo-ille and were moderately severe. Isakeit (1993) had reported that fruit blotch of watermelon is caused by the bacterial pathogen, *A. avenae* subsp. c*itrulli* (*P. pseudoalcaligenes*) which can also cause foliar symptoms. Jett et al. (2002) and Egel (2007) had reported bacterial fruit blotch as a serious disease of watermelons. Bacterial fruit blotch has also been described as a disease of not only watermelon but also other cucurbits (Latin, 2000; Anon, 2008). According to Mohamad Babadoost (2002), bacterial fruit blotch of watermelon, caused by the bacterium *A. avenae* subsp. *citrulli* is an important disease of watermelon and is relatively new in the United States. The disease was first reported in Mariana Islands in 1988 and appeared in commercial watermelon fields in Florida in the spring of 1989. The disease has since been observed in other states but was first diagnosed in Illinois in the summer of 2001. In 1994, the United States had confirmed cases of BFB in at least eleven states and severity ranged from minimal losses in small fields to complete losses of other fields (Anon, 2007).

Both foliar and fruit symptoms were observed in surveyed fields in this study. According to Isakeit (1993), bacterial fruit blotch and leaf spots of watermelon (*C. lanatus)* occur together and cause a lot of damage to the crop in the field. But Martyn et al. (1993) reported that it was curious that where they saw abundant fruit blotch in south Texas fields, they did not find foliar symptoms on the affected plants. Other watermelon growing areas report both leaf and fruit symptoms, so it is likely related to weather conditions. Cultivar or other factors may also affect symptom development. Disease symptoms observed in the course of this study corresponded to what the literature say about bacterial fruit and leaf blotch. However, fruit and foliar symptoms differ greatly. Fruit lesions were reported to enlarge faster on detached fruits than on attached ones in this study. This observation may be related to host nutrition because attached fruits receive nutrients from the parent plant. The disease is reported to be seed-borne (Jett et al., 2002; Anon, 2006; Anon, 2008) and so the first symptoms appear as small water-soaked lesions on seedlings. Leaf lesions are significant reservoirs of bacterium for fruit infection. Some seedlings will collapse and die immediately from infection while some can retain the bacterial infection exhibit symptoms until fruit set (Latin and Hopkins, 1995). Fruit symptoms appear first at flowering and early fruit set as a small water-soaked area less than 1 cm in diameter but it rapidly expands to cover much of the fruit surface in 7 - 10 days (Latin, 2000).

Fruit lesions appear on any portion of the fruit not directly in contact with the soil. This positioning of lesions seems to suggest that the pathogen is air-borne. However, it has been reported that bacterial fruit blotch contamination of seedlings can come from a variety of sources. There is a wide variety of alternate host plants which when growing in close proximity, can transmit bacteria and provide for the spread of BFB and many other diseases to otherwise uninfected plants (Anon, 2008). In severe cases of fruit blotch it was observed in this study that rot also occurred. Jett et al. (2002) had reported that BFB infection does not extend into the meat of the melon, but will cause the rind to rupture, enabling infection by secondary pathogens that cause the fruit to rot. According to them, once the fruit matures and develops a wax layer, it is more difficult for the bacteria to invade the fruit.

In the disease cycle of bacterial fruit blotch, the causal organism, *A. avenae* is seed-borne. The seed may be contaminated externally or internally but there is no evidence of infection within the seed. Contaminated seed results in infected seedlings which serve as secondary inoculum (Anon, 2005). Under excellent environmental conditions either in the greenhouse or in the field the pathogen spreads from diseased to healthy plants. Secondary infections occur through natural openings such as stomata. Leaf lesions serve as a source of inoculum for fruit infection.

Bacteria associated with fruit infection filter down through the flesh, where they become associated with the seed (Anon, 2008). Losses of fresh watermelon to bacterial blotch are direct because they result in reduced quality and quantity or both of the crop (Agrios, 1988). It is therefore, very important to screen seeds before use to avoid planting infested seeds. The “grow out method” is a technique whereby seeds are incubated under greenhouse conditions that are favourable for symptom expression (Anon, 2006). But this technique is laborious and space-consuming. A technique using Polymerase Chain Reaction (PCR) to detect presence of seed-borne BFB has also been reported (Anon, 2008).

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