

**Research article**

Effect of radiation on the incidence and severity of bacterial blight disease induced by *Xanthomonas axonopodis* pv. *Vignicola* on some varieties of cowpea (*Vigna unguiculata* L. Walp)

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Submission: 02/04/2024

Accepted: 02/07/2024

Abstract

Cowpea (*Vigna unguiculata* L.), is a legume that holds great importance in Africa. Around 70% of cowpea production worldwide comes from the arid regions of sub-Saharan Africa, specifically the Savanna and Sahelian areas, where it is primarily cultivated. Nevertheless, this vital crop faces multiple challenges posed by pests and diseases, including a bacterial infection called cowpea bacterial blight (CoBB). It is important to identify resistant sources to develop cowpea varieties that can withstand CoBB, as it has a detrimental effect on cowpea production. The objective of this study was to examine the impact of different radiation treatments on the incidence and severity of the disease. The experiment consisted of eight levels of radiations X-Ray-0Secs (X₀), X-Ray-5Secs (X₁), X-Ray-10Secs (X₂), X-Ray-20Secs (X₃), UV-Rays-0Mins (UV₀), UV-Rays-15Mins (UV₁), UV-Rays-30Mins (UV₂) and UV-Rays-60Mins (UV₃), two cowpea varieties (IT97K-819-118 and Dan'ila) and two levels of bacterial blight inoculum (full inoculum (I₁) and no inoculum (I₀)) combined factorially and laid out in Randomized Complete Block Design with three replications. Disease incidence was determined by assessing the proportion of symptomatic plants as a percentage of the total number of plants per pot, while severity was evaluated using a severity rating scale. The results revealed that disease incidence was highest in the absence of radiation, Danila cultivar, the disease incidence at 0 seconds of X-ray exposure was 82.63%. Similarly, for IT97K-819-118, the disease incidence was 79.33% whereas longer durations of X-ray and UV-ray exposures, particularly 60 minutes of UV-ray exposure, were associated with lower mean values, Danila had a disease incidence of 18.56% (a decrease of 64.07%). IT97K-819-118 exhibited the lowest disease incidence of 28.03% (a decrease of 50.52%). Additionally, IT97K-819-118 demonstrated generally lower severity mean values compared to Danila, when no X-ray radiation was applied, the disease severity for IT97K-819-118 was recorded as 2, indicating slight infection. The study concluded that both X-ray and UV-ray radiation show potential as suppressive agents against cowpea bacterial blight; however, the effectiveness vary depending on the cultivar and the duration of exposure.

Keywords: Cowpea, Radiation Levels, Bacterial Blight, Disease Incidence, Severity

Introduction

Cowpea (*Vigna unguiculata* L.) is a significant legume indigenous to Africa, cultivated in the drier Savanna and Sahelian regions of sub-Saharan Africa (SSA), which contribute approximately 70% of the global cowpea production (Boukar *et al.*, 2018). Besides Africa, cowpea is also grown extensively in Latin America, Southeast Asia, and

the Southern United States (FAO, 2016). Globally, cowpea is cultivated on over 12 million hectares, yielding more than 6.9 million tons of grain annually. Nigeria, Niger, and Brazil are the largest producers of cowpea (FAO, 2016). The grains, leaves, and haulms of cowpea are highly valued for their nutritional content for both humans and livestock, the grains contain approximately 25%

protein, as well as essential macro and micronutrients and the leaves and haulms are primarily used as fodder for livestock and also possess valuable nutrients (Singh, 2006). Despite its adaptability to various regions in SSA, cowpea faces threats from several pests and diseases, including cowpea bacterial blight (CoBB), the first report of this disease dates back to the mid-20th century in the United States (Nandini, 2012). CoBB has since been reported in most countries where cowpea is grown (Bastas and Sahin, 2017; Moretti *et al.*, 2007; Nandini and Kulkarni, 2016). Among the biotic stresses affecting cowpea production in SSA, bacterial diseases such as CoBB and bacterial pustules are particularly severe, leading to significant crop damage (Agbicodo *et al.* and Adegbite *et al.*, 2010).

Improving the sustainability of agriculture while minimizing its environmental impact has become a crucial challenge for humanity in meeting the food demands of a growing global population (Edmondson *et al.*, 2014). The concept of agricultural sustainability involves the development of technologies and practices that do not harm the environment while enhancing food productivity (Pretty *et al.*, 2006). Although chemical compounds are widely used in modern agriculture, the utilization of physical factors presents a promising alternative to increase agricultural yield, improve plant protection, and enhance storage (Aladjadjiyan, 2012). Potential approaches include the application of electromagnetic waves (EWs), magnetic fields (MFs), ultrasound (US), and ionizing radiation (IR). Ionizing Radiation, in particular, has been extensively studied for its ability to enhance the microbiological safety and storability of food, given the concerns regarding food supply safety (Farkas and Mohácsi-Farkas, 2011). The findings from this study will contribute to the identification of effective radiation treatments that can be integrated into breeding programs to develop cowpea cultivars resistant to CoBB and other desirable traits.

Materials and Methods

Experimental Site

The experiment was conducted in the screen house of the International Institute of Tropical Agriculture (IITA) experimental research station Kano. Kano is located in the Sudan Savanna Agro-

ecological zone, latitude 12° 03'N and longitude 08° 31'E and an altitude of 1500 m above sea level (Kowal and Knabe, 1972).

Pot Preparation and Seed Sowing

The sizes of pot for this experiment were 17 cm length and 17 cm breadth plastic pots. The pots were filled with sand mixture after creating a hole at the bottom of the pot to allow passage of water so as not to create a water-logged soil. The pots were then watered and allowed to stand for a day before planting (Kutama *et al.*, 2014). A small hole of 3 cm was dug in each pot and 4 seeds per hole were sown and buried under the soil. Irrigation was used as mode of water supply to the plants for establishment and growth (Kutama *et al.*, 2014).

Exposure of Seeds to Radiation

Seeds of cowpea (Local Dan'ila, and IT97K-819-118) were exposed to radiations (X and UV radiation) at different time periods following the protocols prepared by (Michael and Paul, 2010). Each batch of seeds was placed inside an air and water permeable seed envelope and labelled accordingly. The packed seeds were kept in a desiccator over glycerol (60% by volume) and kept at room temperature for 7 days. This equilibrates the seeds moisture content to 12 to 4%, the ideal moisture condition for achieving efficient induction of mutation. The seeds were then packed into appropriate size petri-dishes and the samples were immobilized by packing with tissue papers. The prepared seeds were then taken to Radiology Department of Rasheed Shekoni Teaching Hospital Dutse, for exposure to irradiation source where the seeds were exposed to X-rays at time periods of 5, 10 and 20 secs. and UV radiation at time periods of 15, 30 and 60 mins., respectively, taking care to observe all safety precautions (Michael and Paul, 2010).

Treatment Combinations and Experimental Design

The experiment was conducted using eight levels of radiations X-Ray-0Secs (X_0), X-Ray-5Secs (X_1), X-Ray-10Secs (X_2), X-Ray-20Secs (X_3), UV-Rays-0Mins (UV_0), UV-Rays-15Mins (UV_1), UV-Rays-30Mins (UV_2) and UV-Rays-60Mins (UV_3), two cowpea varieties (IT97K-819-118 and Dan'ila) and two levels of bacterial blight

inoculum (full inoculum (I₁) and no inoculum (I₀)) combined factorially giving a total of (8×2×2=32) 32 treatments combination and laid out in Randomized Complete Block Design with three replications (Zar, 2010).

Inoculum Sample Collection

The technique of (Moretti *et al.*, 2007) was adopted with little modification for inoculum sample collection. Diseased leaf samples of Cowpea showing symptoms of common bacterial blight disease was collected from a farm considered as a 'hot spot' for many years in the International Institute of Tropical Agriculture (IITA) cowpea research farm at Minjibir, Kano State. The leaves were then kept in plastic bags and taken to the Centre for dry land Agriculture, Bayero University Kano.

Isolation and Identification of the Inoculum

The leaves were surface sterilized with 0.5% NaOCl, rinsed three times with sterile distilled water and dried under shade for 7 days. A portion of leaf (1–2 mm) with bacterial infection was placed on nutrient agar (NA). The plates were then incubated at 28°C for 48 to 2 hours. Sub-culturing was performed to obtain pure cultures. The isolate was then stored on NA slants for further use (Duche *et al.*, 2015).

Gram test of the isolates following the Gram staining procedure of Schaad, (2001) was done to determine the morphological characteristics. Culturally, yellow colony indicates the presence of the bacteria (*Xanthomonas campestris* pv. *Vignicola*) and microscopically gram-negative rod shaped confirms the presence of the bacteria (Tika and Sundar, 2009).

Preparation and Application of Inoculum

Inoculum suspensions were prepared by harvesting bacterial cells from petri-dishes into sterilized deionized distilled water. Suspensions were adjusted turbidimetrically using McFarland standards. McFarland standards was used as a reference to adjust the turbidity of the bacterial suspension so that the number of bacteria will be

within a given range to standardize the approximate number of the bacteria in a liquid suspension by visually comparing the turbidity of the harvested test suspension with that of a McFarland standard. Six hours prior to the inoculation plants were misted with tap water from 10 a.m. to 4 p.m. to create a favorable environment for disease development, plants were inoculated 14 days after planting (dap). In the screen house, the average temperature was 24.0 °C and 25.5 °C for the experiment. The average relative humidity in the screen house was 88%. The isolated bacterial suspensions were used to inoculate the growing seedlings using spray pump. The inoculum was poured into the spray pump and was introduced to the leaves by spraying the leaves until it dried out, after inoculation, plants were covered with polyethene bags for 48hrs to increase humidity in plant canopy to enhance the establishment infection (Agbicodo *et al.*, 2010).

Pathogenicity Test

Pathogenicity test was conducted in a screen house using Koch's postulate. Steam-sterilized soil in plastic containers, a total number of eight plastic containers was used: six plastic containers for the tested organism, *Xanthomonas*, and two plastic containers for the control. Five 5kg of soil was placed in each of the plastic containers. Three (3) seeds of the cowpea were planted in each container. Two weeks after planting, inoculum prepared from the isolate were sprayed on young, healthy cowpea seedlings at the upper and lower surfaces of the leaves till run-off, covered with a polythene for 48hrs. The presence or absence of characteristic symptoms was observed.

Data Collection

Determination of Disease Incidence

The incidence of the bacterial blight disease was recorded by establishing the proportion of plants showing the symptoms and expressing the result as percentage of total number of plants per pot as suggested by Kutama *et al.* (2013a) and Fagwalawa *et al.* (2013).

$$\text{Disease Incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants visited}} \times 100$$

Determination of Disease Severity

The severity was scored at two (2) weeks after inoculation. The severity percentage was

$$\text{Disease severity (\%)} = \frac{\text{Area of affected leaf}}{\text{Total leaf area}} \times 100$$

0-0% 0 = No infection

1-10% 1 = very slight infection (very few spots on the leaves and a few leaves affected)

11-20% 2 = Slight infection (few spots on the leaves and more visible)

21- 40% 3 = moderate infection (up to four spots per plant and general light spotting i.e appears clearly).

41- 60% 4 = Severe infection (Nearly every leaf with lesions, plant still remaining normal form)

61 – 100% 5 = Lead to death (only few leaves left green, most leaves are dead) (Kutama et al., 2011).

Statistical Analysis

Data collected were subjected to Analysis of Variance (ANOVA) using augmented RCBD R package version 0.1.5.9000 and significantly

calculated using the formula below and then the severity was scored on the infected plant using the severity rating scale as follows:

different means were separated using LSD post hoc test ($P \leq 0.05$).

Results and Discussion

Pathogenicity Test

The results showed initial appearance of water-soaked spots on leaves, which enlarge to irregular brown necrotic lesions surrounded by yellow haloes, and adjacent lesions frequently coalesced. This has tallied with the report Kutama et al. (2018) on the symptoms of bacterial blight in cowpea. The re-isolated pathogen from inoculated plants was found to be identical to the isolate used for inoculation and the same bacteria species (*Xanthomonas axonopodis*) was identified. Control plants did not show any symptom of common bacterial blight disease (Kutama et al. 2018).

Table 1: Pathogenicity Test Result for Bacterial Blight Pathogen of (Danila and IT97K-819-118) Cowpea varieties

S/No	Bacterial Isolate	Pathogenicity
1.	<i>Xanthomonas axonopodis</i>	Positive (+)

Effects of Different Radiations on the Disease Incidence (%) of *Danila* and *IT97K-819-118* Cowpea (*Vigna Unguiculata*) Cultivars.

Table 2 presents the effects of different radiation exposures on the disease incidence in two cowpea cultivars, Danila and IT97K-819-118. The results from Analysis of variance (ANOVA) indicated that the differential effect of eight levels of radiations was highly significant ($P < 0.05$) in diseases incidence.

The results of this study demonstrate that different radiation treatments have varying effects on disease incidences and disease severity of the two

cowpea cultivars: Danila and IT97K-819-118. The disease incidence decreased significantly with increasing durations of X-ray exposure for both cultivars. The Danila cultivar had a higher baseline disease incidence compared to IT97K-819-118. However, both cultivars exhibited a decreasing trend in disease incidence with longer exposure times. UV-ray exposure: Similar to X-ray exposure, disease incidence decreased significantly with increasing durations of UV-ray exposure for both cultivars. The baseline disease incidence was higher in Danila compared to IT97K-819-118.

Table 2: Effects of Different Radiations on the Disease Incidence (%) on *Danila* and IT97K-819-118 Cowpea (*Vigna Unguiculata*) Cultivars.

Radiations	Varieties	
	Dan'ila	IT97K-819-118
X-RAY-0Secs	82.63±0.8 ^a	79.33±0.8 ^a
X-RAY-5Secs	74.30±0.4 ^a	53.33±0.8 ^b
X-RAY-10Secs	33.16±0.2 ^c	33.27±1.6 ^{c,g}
X-RAY-20Secs	30.72±0.8 ^{b,c}	33.17±1.4 ^{d,c}
UV-RAY-0Mins	76.30±0.8 ^a	56.60±0.7 ^e
UV-RAY-15Mins	57.14±1.6 ^d	40.11±0.0 ^{b,c}
UV-RAY-30Mins	39.80±0.8 ^{e,c}	26.30±0.4 ^s
UV-RAY-60Mins	18.56±0.8 ^f	28.03±0.7 ^{h,d}
P-Value =0.000		

Means followed by the same letters in the column are not significantly different at P<0.05 alpha level.

Effects of Different Radiations on the Cowpea Blight Disease Severity (%) on *Danila* and IT97K-819-118 Cowpea (*Vigna Unguiculata*) Cultivars.

Table 3 presents the severity of the disease recorded for each cultivar under different radiation durations. In the case of the Danila cultivar, exposure to X-ray radiation for different durations showed a consistent pattern. When no radiation (0 secs) was applied, the disease severity was recorded as 2, indicating slight infection. However, with an increase in radiation duration to 5 secs, 10 secs, and 20 secs, the disease severity decreased to 1, indicating very slight infection. Regarding UV-ray radiation, the Danila cultivar displayed similar trends.

Effects of Different Radiations on Disease Severity: X-ray exposure: Disease severity showed a consistent pattern of decrease with increasing durations of X-ray exposure for both cultivars. The severity decreased from slight infection to very

slight infection for all exposure durations. UV-ray exposure: Disease severity also decreased with longer durations of UV-ray exposure. However, an interesting observation was made for IT97K-819-118, where the disease severity decreased to no infection at 30 minutes of exposure but reverted to very slight infection at 60 minutes.

Comparing the two cultivars, IT97K-819-118 generally exhibited lower disease incidences and lower disease severities than Danila, indicating a higher level of disease resistance. Overall, the results demonstrate that both X-ray and UV-ray radiations had a significant impact on reducing disease incidences and severities values for cowpea blight in both cultivars. Longer exposure durations generally resulted in greater reductions in disease parameters. The differences observed between the two cultivars suggest variations in their responses to radiation and potential differences in disease resistance.

Table 3: Effects of Different Radiations on the Disease Severity (%) of Cowpea Blight on *Danila* and IT97K-819-118 Cultivars.

Radiations	Varieties	
	Dan'ila	IT97K-819-118
X-RAY-0Secs	2.00	2.00
X-RAY-5Secs	1.00	1.00
X-RAY-10Secs	1.00	1.00
X-RAY-20Secs	0.00	1.00
UV-RAY-0Mins	2.00	1.00
UV-RAY-15Mins	1.00	1.00
UV-RAY-30Mins	1.00	0.00
UV-RAY-60Mins	1.00	1.00

Key: 0= No Infection, 1= Very slight Infection (1-10%), 2= Slight Infection (11-20%)

The interaction effect of Varieties, Radiation Levels and Inoculum on Disease Incidence and Disease Severity.

Table 4 provides information on the effects of different treatment combinations of varieties, radiations, and inoculum on mean values of Disease Incidence and Disease Severity. The disease incidence means are presented as percentages, with lower percentages indicating a lower incidence of the disease. Upon examining the data, it becomes apparent that the disease incidence means vary across the treatment combinations. The highest disease incidence means of 100.00% were observed in the following combinations: Danila: UV₀:I₁, Danila: X₀:I₁, and IT97K-819-118: X₀:I₁. These combinations indicate a high susceptibility to the disease, as they had the highest disease incidence means. On the other hand, some treatment combinations demonstrated significantly lower disease incidence means. For example, combinations such as IT97K-819-118: UV₂:I₁, Danila: UV₃:I₁, and Danila: X₃:I₁ exhibited disease incidence means of 16.50%, 33.50%, and 33.50%, respectively. These combinations suggest a relatively effective resistance to the disease.

Additionally, there are treatment combinations that showed intermediate disease incidence means, falling between the highest and lowest values. Combinations like Danila: X₂:I₁, Danila: UV₁:I₁, and IT97K-819-118: UV₁:I₁ had disease incidence means of 83.50%, 66.50%, and 66.50%, respectively. These combinations indicate a moderate level of disease incidence. The interaction effect of varieties, radiation levels, and inoculum on disease incidence and disease severity is shown in Table 4. Disease Incidence: The disease incidence means vary across the treatment combinations. Combinations such as Danila: UV₀:I₁, Danila: X₀:I₁, and IT97K-819-118: X₀:I₁ had the highest disease incidence means (100.00%), indicating high susceptibility to the disease. Combinations like IT97K-819-118: UV₂:I₁, Danila: UV₃:I₁, and Danila: X₃:I₁ exhibited lower disease incidence means (16.50%, 33.50%, and 33.50% respectively), suggesting effective resistance to the disease. Combinations like Danila: X₂:I₁, Danila: UV₁:I₁, and IT97K-819-118: UV₁:I₁ had intermediate disease incidence means (83.50%, 66.50%, and 66.50% respectively),

indicating a moderate level of disease incidence. The treatment combination IT97K-819-118: UV₂:I₁ performed the best in terms of disease incidence means, with the lowest value of 16.50%, suggesting effective resistance against the disease. Disease Severity: The severity means of different treatment combinations are grouped. Combinations like IT97K-819-118: X₀:I₁, Danila: X₀:I₁, and IT97K-819-118: UV₀:I₁ demonstrated relatively higher disease severity. Combinations like Danila: UV₀:I₁, Danila: X₁:I₁, IT97K-819-118: UV₁:I₁, and IT97K-819-118: X₁:I₁ showed decreasing disease severity means, indicating increasing effectiveness of the treatments. Combinations like Danila: X₃:I₁ exhibited the lowest disease severity among all combinations. Combinations involving Danila and different inoculum levels tended to show better performance in terms of disease severity. Combinations including IT97K-819-118 and various radiation levels also exhibited favourable results.

Therefore, the radiations are playing a big role on the progress of the disease, where it suppresses the inoculum performance. In general, longer exposure times to X-ray radiation resulted in decreased disease severity in both cultivars. This suggests that X-ray radiation may have a suppressive effect on the progression of cowpea blight disease. However, it's important to note that the effect of X-ray radiation on disease severity may be cultivar-specific, as the IT97K-819-118 cultivar consistently exhibited lower values than Danila across all X-ray radiation treatments. The effects of UV-ray radiation on disease severity were more complex. For both cultivars, increasing exposure times to UV-ray radiation generally led to a decrease in disease severity. This indicates that UV-ray radiation may have a suppressive effect on cowpea blight disease. However, the longest exposure time of 60 minutes (UV-RAY-60Mins) resulted in an increase in values compared to the previous treatment for both cultivars. This unexpected result suggests that prolonged exposure to UV-ray radiation may have a detrimental effect on disease severity in certain circumstances. Our results were supported by Ikram *et al.*, (2015) that there was complete suppression of root rot fungi when seeds were treated with x-rays for 5, 10 and 20 sec.

Table 4: The interaction effect of Varieties, Radiation Levels and Inoculum on Disease Incidence, Disease Severity and Area under Disease Progress Curve of *Danila* and IT97K-819-118 Cowpea (*Vigna unguiculata*) Cultivars.

Treatments Combination	DI (%)	DS (%)
IT97K-819-118: X ₀ :I ₁	100.00 ^a	3.67 ^a
Danila: X ₀ :I ₁	100.00 ^a	3.33 ^{ab}
IT97K-819-118: UV ₀ :I ₁	100.00 ^a	3.33 ^{ab}
Danila: UV ₀ :I ₁	83.00 ^{ab}	3.00 ^{abc}
Danila: X ₁ :I ₁	66.50 ^{abc}	2.33 ^{bcd}
IT97K-819-118: UV ₁ :I ₁	66.50 ^{abc}	2.33 ^{bcd}
IT97K-819-118: X ₁ :I ₁	66.50 ^{abc}	2.33 ^{bcd}
IT97K-819-118: UV ₃ :I ₁	66.50 ^{abc}	2.00 ^{cde}
Danila: UV ₂ :I ₁	66.50 ^{abc}	1.67 ^{de}
Danila: X ₂ :I ₁	66.50 ^{abc}	1.67 ^{de}
Danila: UV ₁ :I ₁	66.50 ^{abc}	1.00 ^{ef}
Danila: UV ₃ :I ₁	50.00 ^{bcd}	1.00 ^{ef}
IT97K-819-118: UV ₂ :I ₁	50.00 ^{bcd}	1.00 ^{ef}
IT97K-819-118: X ₂ :I ₁	33.00 ^{cde}	1.00 ^{ef}
IT97K-819-118: X ₃ :I ₁	33.00 ^{cde}	1.00 ^{ef}
Danila: X ₃ :I ₁	16.50 ^{de}	0.33 ^f
Danila: UV ₀ :I ₀	0.00 ^e	0.00 ^f
Danila: UV ₁ :I ₀	0.00 ^e	0.00 ^f
Danila: UV ₂ :I ₀	0.00 ^e	0.00 ^f
Danila: UV ₃ :I ₀	0.00 ^e	0.00 ^f
Danila: X ₀ :I ₀	0.00 ^e	0.00 ^f
Danila: X ₁ :I ₀	0.00 ^e	0.00 ^f
Danila: X ₂ :I ₀	0.00 ^e	0.00 ^f
Danila: X ₃ :I ₀	0.00 ^e	0.00 ^f
IT97K-819-118: UV ₀ :I ₀	0.00 ^e	0.00 ^f
IT97K-819-118: UV ₁ :I ₀	0.00 ^e	0.00 ^f
IT97K-819-118: UV ₂ :I ₀	0.00 ^e	0.00 ^f
IT97K-819-118: UV ₃ :I ₀	0.00 ^e	0.00 ^f
IT97K-819-118: X ₀ :I ₀	0.00 ^e	0.00 ^f
IT97K-819-118: X ₁ :I ₀	0.00 ^e	0.00 ^f
IT97K-819-118: X ₂ :I ₀	0.00 ^e	0.00 ^f
IT97K-819-118: X ₃ :I ₀	0.00 ^e	0.00 ^f
LSD	0.80	1.00

Means followed by the same letters in the column are not significantly different at P<0.05 alpha level.

Brown *et al.* (2001) also reported that seed treatments with low doses of UV were used to elicit host resistance to black rot in cabbage (*Brassica oleracea* L.). It is important to note that the combinations involving Danila and different inoculum levels tend to show relatively better performance in terms of disease severity. Additionally, the combinations including IT97K-819-118 and various radiation levels also exhibit favourable results.

Conclusion

The research revealed that, the significant impact of radiation treatments on reducing disease parameters in cowpea blight. Both X-ray and UV-ray radiation show potential as suppressive agents against the disease. However, the effects may vary depending on the cultivar and the duration of exposure.

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