

# Development of wet rot disease of *Amaranthus cruentus* L. caused by *Choanephora cucurbitarum* (Berk. and Rav.) Thax. in response to phytochemical treatments and inoculation methods

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## ABSTRACT

The development of the wet rot disease of *Amaranthus cruentus* L. caused by *Choanephora cucurbitarum* (Berk. and Rav.) Thax. in response to treatment of the crop with leaf extracts of *Dennettia tripetala* (Baker f.), *Spondias mombin*, *Bryophyllum pinnatum* (Lam.) Oken, and methods of inoculation of the organism, were evaluated in the greenhouse of the Department of Plant Health Management of Michael Okpara University of Agriculture, Umudike. The experiment was a 3 × 5 factorial in a Completely Randomized Design (CRD) replicated five times. The treatment comprised three methods of inoculation: un-inoculated seeds, inoculated seeds and inoculated plants at six weeks after planting and fungicide products. The fungicides were Benomyl (a synthetic fungicide, plant extracts) and untreated control using sterile water. The results indicated that the highest infection rate of the disease was obtained in the first six weeks of inoculation of the organism, with plants in the control pots having the highest disease severity (9.8). Among the plant extracts used, extracts of *D. tripetala* gave the best control with a severity of 5.8. Plants in pots where the seeds were inoculated with *C. cucurbitarum* and those in pots of un-inoculated seeds had less damage, performed better and produced higher dry matter yield than plants in pots where the seedlings were inoculated with the pathogen. There was a significant ( $P < 0.05$ ) difference in the growth and yield of plants treated with Benomyl and plant extracts, compared with those of the control.

**Keywords:** Phytochemicals, *Amaranthus cruentus*, *Choanephora cucurbitarum*, severity score, incidence, disease.

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## INTRODUCTION

*Amaranthus cruentus* is a vegetable crop, belonging to the family Amaranthaceae. It was originally domesticated in central Africa and Mexico. This annual with a short life cycle may reach a height of 15 to 20 cm (Raemaekers, 2001). The small seeds of *Amaranthus* may be sown directly and harvested 30 to 50 days after sowing (Tindal, 1986).

Compared to other leafy vegetables *Amaranthus* is remarkably rich in Vitamins A, C and other minerals which include iron, calcium, folate, and amino acids with high levels of sulphur (PROTA, 2004). One hundred grams contain 76% water, 4.6% protein, 1.8 g cellulose,

410 mg calcium, 8.9 mg iron, 5.7 mg beta-carotene, and 64 g vitamin C (Raemaekers, 2001).

It can be ground into powder and mixed with wheat and used in confectionaries. *Amaranthus* is a rich food, with medicinal properties for children, lactating mothers and patients with constipation, fever, hemorrhage, anaemia, or kidney complaints. *Amaranthus* is the largest source of nutrients of all vegetables that can be grown in tropical Africa.

*Amaranthus* production has been reduced by pest and disease attack. It is mostly affected by fungal diseases like, damping off caused by *Pythium* spp., stem canker by

*Rhizoctonia* spp., *Alternaria* leaf spot, wet rot caused by *Choanephora cucurbitarum* (PROTA, 2004). The wet rot of *Amaranthus* causes a lot of damage if ignored, especially in the endemic areas (Robert et al., 2003). *C. cucurbitarum* affects portions of the stem which are cut during harvesting (Messiaen, 1994). There is a high incidence of the disease in Nigeria and this adversely affects the cultivation of *Amaranthus* (Odebunmi-Oshilanu, 1977).

Raemaekers (2001) reported that year round cultivation of *Amaranthus* is common practice in Africa including coastal regions of Nigeria, Democratic Republic of Congo (DRC) and Benin Republic. In the humid tropics of Nigeria wet rot of *amaranthus* reduces productivity of the crop (Awurum and Ogbonna, 2013). PROTA (2004) indicated that wet rot of *amaranthus* induce by *C. cucurbitarum* causes a lot of damage to the crop. Most farmers use pesticides like Dithane M-15 and Benlate to control the disease, but these pesticides leave residues on the crops and may cause poisoning to consumers and the farmers involved in the application of these pesticides (FAO, 2000). Due to the hazards of pesticides, the use of plant extracts to protect vegetables is preferable in controlling fungal diseases (Awurum and Nwaneri, 2011; Awurum and Ogbonna, 2013). This is because they are not as toxic to man. Also, plant extracts are easier to obtain, cheaper and accessible to farmers (Enyiukwu and Awurum, 2012).

The objective of this study therefore, is to determine the effect of using leaf extracts of *Dennettia tripetala*, *Spondias mombin*, *Bryophyllum pinnatum*, and a synthetic fungicide on the severity of the wet rot of *Amaranthus* caused by *Choanephora cucurbitarum* and performance of the crop.

## MATERIALS AND METHODS

### Plant material and cropping conditions

Greenhouse experiments were carried out at the Department of Plant Health Management Michael Okpara University of Agriculture, Umudike, Nigeria, located on latitude 5°29' N, longitude 7°33' E and altitude of 122 m above sea level with an annual rainfall of 1916 mm, relative humidity 76% and temperature range of 19 to 35°C (NRCRI, 2010). All the experiments were performed on 10-L buckets filled with 7 kg of a sterilized (Blodgett and Swart, 2002) soil rich in loam. Day and night room temperature of 27 and 25°C and 78 to 82% were maintained. Plants were watered daily (200 ml/pot). A 3 × 5 factorial experiment was performed in a Completely Randomized Design.

### Fungal maintenance and inoculation procedures

*C. cucurbitarum* was recovered from naturally infected *Amaranthus* plants at the Agricultural Experimental Farm - Michael Okpara University. Diseased tissues were cut into small pieces and superficially sterilized for 3 min by immersion in a 75% ethanol solution and then thoroughly rinsed three times with sterile distilled water. The tissues were incubated in a moist chamber (autoclaved

Petri plates containing wet Whatman No. 1 filter paper) for five days at 27°C temperature dark/light). The developed fungal colonies were transferred on Petri dishes containing Potato Dextrose Agar (PDA) and incubated 3 to 5 days at 27 ± 2°C for spore production.

A suspension of 1 × 10<sup>5</sup> spores per ml was prepared in sterile distilled water. *A. cruentus* seeds, collected at the Department of Plant Health Management, were soaked for three hours in the *C. cucurbitarum* spore suspensions. Control seeds were treated with sterile distilled water.

*A. cruentus* seedlings were inoculated by spraying 20 ml of spore suspension. The tissues of the plant were exposed to the inoculum by slightly pressing with sand paper. After spraying, plants were covered with polythene foil for 72 h to create an environment conducive for *C. cucurbitarum* growth. Seedlings treated with sterile water were used as an untreated control.

### Extracts preparation and treatments

Leaves of *Dennettia tripetala*, *Spondias mombin*, and *Bryophyllum pinnatum* were collected and air dried for 3 to 10 days. For each species, 20 g of dried material was extracted with 100 ml of sterile water following the procedures described by Amadioha (2004).

Sets of inoculated and un-inoculated seeds were treated by soaking in a solution of 0.2 g (100 per ml<sup>-1</sup>) of Benlate or in a solution of each plant extracts. Seeds soaked in sterile water were used as an untreated control. After 1 h treatment seeds were dried for 1 h and sowed.

Plant height, number of leaves, leaf area (using leaf area meter) and disease severity were recorded at two weeks interval starting four weeks after planting. Stem girth (using veneer calliper) was recorded at the 6<sup>th</sup> week. Plants and inflorescences dry matter were recorded at maturity. Disease reaction was evaluated on each seedling by recording the severity of visible external stem girth, as well as the yield components, symptoms at 3, 6, 9 and 12 days after inoculation as (James, 1983):

$$\text{Disease severity} = \frac{\text{Sum of Individual Disease Ratings}}{\text{Total number of plants examined}}$$

A modified Allen et al. (1981) scale was used to record the disease severity ratings: 1 = no symptoms; 2 = lesion present on less than 20% of the plant shoot; 4 = lesion present on less than half of the tissue; 6 = lesion present on up to 60% of the plant shoot; 8 = lesion present on most of the plant shoot; 10 = heavy lesion on plant shoot, heavy defoliation occurs.

Statistical significance was determined by variance analysis (ANOVA) and means were separated using Fisher's Least Significant Difference (F-LSD) at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

Results obtained on the growth and yield of *Amaranthus* during the growth period were presented in Table 1. Generally, there were significant differences ( $P < 0.05$ ) between the various treatments. Both free and inoculated seeds performed significantly better than the plants that were inoculated 6 weeks after planting (WAP) in terms of growth parameters, plant height, leaf area, number of leaves, stem girth, as well as the yield components, dry matter yield of the plant and dry matter yield of the inflorescence. This showed that the effect of the pathogen was more when the seedlings were inoculated

**Table 1.** Growth and yield parameters registered on *Amaranthus cruentus* plants obtained from Inoculated seed (I) and seedling(s) with *C. cucurbitarum* ( $1 \times 10^5$  spore per ml) and not inoculated (-) seeds and treated with: *Dennettia tripetala* (DT), *Spondias mombin* (SM) or *Bryophyllum pinnatum* (BP) leaf extract, Benlate (BY) or Sterile water (SW).<sup>a</sup>

Treatment	Plant height (cm)	Leaf area (cm <sup>2</sup> )	Number of leaves	Stem girth (mm)	Dry weight of inflorescence	Dry matter weight of plant
ISM	69.28	118.44	14.80	1.60	3.71	12.33
-SM	70.00	121.76	16.20	1.34	3.38	12.30
SSM	62.98	112.44	15.00	1.24	0.21	2.70
IDT	91.00	148.36	22.60	1.30	4.22	14.92
-DT	91.50	148.60	22.60	1.72	4.27	13.18
SDT	85.96	134.52	19.80	1.42	0.23	3.55
IBP	74.44	124.32	16.00	1.74	2.26	11.25
-BP	72.58	120.12	16.80	1.40	2.12	11.30
SBP	70.12	118.80	15.40	1.22	0.15	0.86
IBY	81.50	131.80	22.40	1.70	4.87	11.48
-BY	91.00	146.24	25.80	1.72	5.56	17.32
SBY	86.28	136.78	19.60	1.38	1.14	6.14
ISW	83.26	132.10	19.60	1.52	2.12	11.28
-SW	74.54	121.28	16.00	1.36	2.28	11.03
SSW	76.04	124.68	15.80	0.76	0.04	0.36
LSD	2.40	4.36	2.32	0.109	0.133	0.218

<sup>a</sup> Values are averages of five plants.

than the inoculation of seeds as there was an insignificant ( $P > 0.05$ ) effect of the pathogen on the plants whose seeds were inoculated (Figures 1 and 2). Consequently, plants from such seedlings had poor yield.

The results also showed that there were significant differences between plants treated with the various plant extracts and Benomyl. The performance of plants treated with the synthetic fungicides was significantly ( $P < 0.05$ ) better than those of other treatments. The least performance was observed from plants treated with sterile water, which acted as the control. The trend was the same with the crop yield, as both the plants treated with the extracts and those treated with Benomyl yielded better in terms of dry matter weight of plants and dry matter weight of the inflorescence, than the control. According to Okwu and Josiah (2006), the presence of phenolic compounds in *B. pinnatum* indicates that it may be an anti-microbial agent. This was further confirmed by the foregoing results.

The effect of wet rot of *Amaranthus* caused by *C. cucurbitarum* on artificial inoculation in the green house is shown in Figure 3. There were significant ( $P < 0.05$ ) differences between the disease severity of wet rot on plant treated with Benomyl (4.3) and other plant extracts. Between plants treated with plant extracts, those treated with extracts from *D. tripetala* (5.8) had the least disease severity which also translated into better yield compared with plants treated with extracts of *B. pinnatum* (7.9) and *S. mombin* (7.8). Generally, the control plants had the higher disease severity (9.8) compared with the chemical treatment. It therefore shows that both the plant extracts

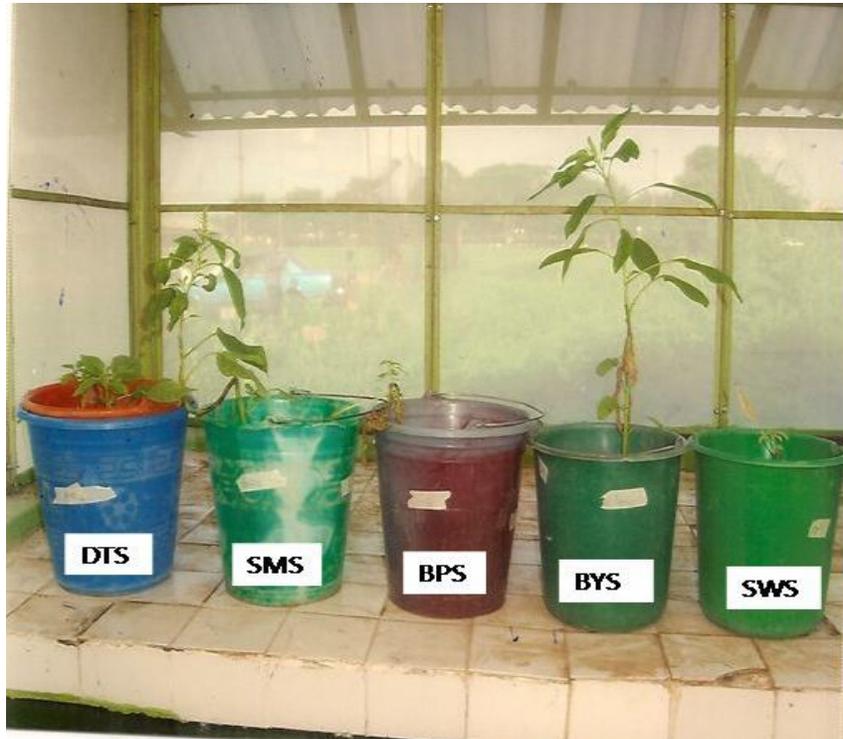
and the synthetic fungicide were effective in the control of the disease. Osunlaja and Bello (1992) reported satisfactory control of the wet rot disease of *Amaranthus* using Neem. Plant extracts are preferably secure; they are cheap and easily accessible (Ofuya and Salami, 2001; Amadioha, 2004; Awurum et al., 2005; Stephen, 2006; Oluma and Elaigwu, 2006).

Figure 4 also showed that the disease severity increased considerably faster within the first 6 days of inoculation with all the treatments after which severity tended to slow down and become gradual, indicating that the greatest damage caused by the pathogen occurs within the first few days of infection (Robert et al., 2003). It is proper therefore that any control measure would have to be applied as early as possible at the onset of the disease.

These results showed that free or inoculated seeds were less affected by wet rot than inoculated seedlings. This agrees with the work carried out by Osunlaja and Bello (1992) and Robert et al. (2003) on the effect of *C. cucurbitarum* on crops. This consequently caused the reduction in both growth and yield of the *Amaranthus*.

## Conclusion

From this study, it was observed that plants raised from free or inoculated seeds of *Amaranthus cruentus* were not affected by the pathogen *C. cucurbitarum*, but the plants sprayed and inoculated artificially were affected. This resulted in a reduction in the growth and yield of



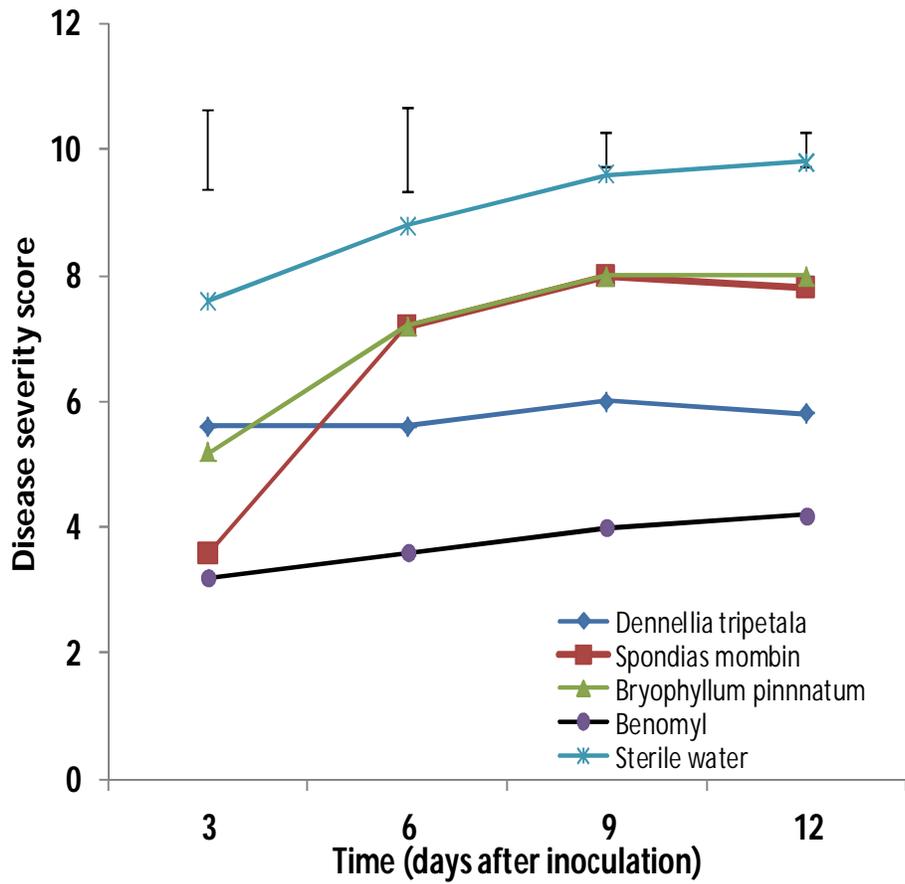
**Figure 1.** *Amaranthus cruentus*, 2 weeks after artificial inoculation with *Choanephora cucurbitarum* and sprayed with various chemicals and sterile water in the greenhouse.



**Figure 2.** Response of *Amaranthus cruentus* treated with sterile water and different inoculation methods.



**Figure 3.** Wet rot disease induced by *C. cucurbitarum* on artificially inoculated *Amaranthus* in the greenhouse.



**Figure 4.** Progress curves of the disease severity of *Amaranthus cruentus* induced by artificially inoculated *C. cucurbitarum*.

Amaranthus in the green house.

The plant extracts and synthetic fungicide Benomyl used in controlling the disease induced by the pathogen were effective compared to the control. The effectiveness of the different plant extracts was significant, with *D. tripetala* giving the best control of the disease. This indicates that the plant extracts are effective in the control of the wet rot of Amaranthus which translated to a better yield of the crop.

Considering the effectiveness, availability and lack of reasonable cost, ease of application and compatibility with the environment, these plant derived extracts have the potential to replace the synthetic chemicals that have adverse effects, in the control of wet rot of Amaranthus. Farmers should use these extracts at the onset of the disease. This is consequent upon the fact that the pathogen has high infection rate within the first week of infection, especially when the environmental condition are conducive for disease development.

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