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Effects of damping-off fungus on seed germination of amaranth (*Amaranthus Hybridus*)

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Abstract

Seed borne pathogens are microorganisms that can prevent seed germination and cause serious threats to seedling development in the field. The research was conducted on the effects of damping off fungus on seed germination of Amaranth (*Amaranthus hybridus*) collected from some Agricultural farmland in Lapai Local Government Area of Niger State, North central Nigeria. The isolation of fungal species from *Amaranthus hybridus* seeds of the infected plants having symptoms of damping off disease using agar plate method and was identified using the morphological characteristics features from mycological atlas. Isolation from the infected seeds revealed that *Rhizoctonia solani* were associated with the plants. Seed weight (mg) and seed germination (%) were determined using the international rules for seed testing and the physiological seed quality indices were determined; these are germination index (GI), germination rate index (GRI) and seedling vigor index were arranged in Randomized Complete Block Design (RCBD) all in triplicates. The result indicated that seeds from uninfected plants recorded seed weight (8.97mg) as compared with the infected seeds (5.21mg), there was significantly higher germination (98.67%) observed in uninfected seeds compared with seeds of *Amaranthus* plants that were infected (32.13%) with damping off fungus. Germination index was observed to be higher in seeds of uninfected plants (9.34) and lower in seeds of infected plants (4.34). The significant effects observed of the fungus on both seed and seedlings are highly virulence on the infected plants. The research revealed that a very good association between *Rhizoctonia solani* causing damping off disease and seed germination failure of *Amaranthus hybridus* plants. In the cultivation of *Amaranthus hybridus*, seed weight, and physiological seed quality attributes should be considered for an effective damping off disease suppression.

Keywords: Germination index; *Rhizoctonia solani*; Seed weight; Vigor index; Virulence

1 Introduction

Damping-off disease in Amaranth, induced by the fungus, severely disrupt the early stage of the crop establishment by causing poor emerging of the seed and death of the emerging seedling, which result in poor stand in seedbed and field (15). Seed may also rot before germination because of fungi infection (1). Often affected seedlings emerging from the soil level which have developed watered-soaking, shriveling of the stem at the soil level; then they fall over completely and die. Several factor including poor soil drainage resulting into continuous wetness and low soil temperature considerably predisposes the crop to the disease (16).

Damping-off was considered "the most serious problem encountered in raising nursery seedlings and consequently was one of the most focused research areas since the beginning of its description (12, 16). Many authors refer to damping-off as a "disease" (23), while others refer to damping-off as a "symptomatic condition" (7, 24). In the former case, damping-off is usually associated to soil-borne pathogens while in the latter case, seed-borne pathogens can promote damping-off. Microorganisms affect amaranth seeds during germination and growing state thereby causing Damping-

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off disease. It can be characterized by killing or weaken of the seed or seedlings before they germinate. If the seedlings do manage to germinate, they may not emerge, or they may emerge and then become brown, pinched, and die. Other symptoms include stunted and yellow growth, or reduced root growth with brown spots on the roots and coleoptile. The lack of high quality seeds and the prevalence of seed borne organisms are the main constraints in maintaining the *Amaranthus hybridus* production. (9, 11) reported that more than 400 seed borne diseases in 72 crops inflicting an estimated yield loss amounting to around 200 million US dollars annually.

The lack of high quality healthy seeds and the prevalence of seed borne diseases such as damping off diseases are among the main constraints in maintaining the sustainability of Amaranth vegetable production and per capital consumption. Seeds of vegetables are more vulnerable to attack by seed and soil borne inducing pathogens and can result to damping off of seedlings. Due to the prevalence of these biotic factors, the main aim of the study is to determine the effects of some damping off inducing pathogens on seed germination of *Amaranthus hybridus* under greenhouse condition.

2 Material and methods

2.1 Experimental Location

Pots experiment was conducted under greenhouse condition of the department of biological science, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria. Located in the north central (middle belt) zone of Nigeria. The study area is situated in the rainforest vegetation zone of south-western Nigeria on latitude 713, 49.46^0N. Longitude 326, 11.98^0E and Altitude of 98m above sea level. The climate is humid with a mean annual rainfall of 1003mm, annual temperature range of 31.9 to 34.8ºC and humid from 79.7 to 90.1%.

2.2 Samples Collection

Seeds of *Amaranthus hybridus* was collected inside sterile paper envelopes from infected plants in Ibrahim Badamasi Babangida University Agronomic farm, Lapai, Niger State. The samples were stored in the refrigerator at room temperature till the seeds were used for the further experiments.

2.3 Isolation and identification of seed borne fungus

Twenty (20) seeds of *Amaranthus hybridus* were plated on the potato dextrose agar (PDA) in petri dishes and incubated for 7 days at 28±2 ^{o}C (ambient temperature) on laboratory bench top. Each treatment was replicated three times. The petri-dishes were observed for growth of associated fungi and pure culture of the isolated microorganisms were obtained by repeated sub-culture. Identification of the isolated fungus was compared using the morphological and microscopic features of the isolated microorganisms using the standard mycological identification key of Barnett and Hunter (4). The percentage frequency of occurrence of fungal species isolated calculated thus:

$$\text{Percentage frequency } \% = \frac{\text{Total number of occurrences of the organisms}}{\text{Total number of observation}} \times 100$$

2.4 Seed weight

Seed weight was determined by the use of 100 seed weight method of International Rules for Seed Testing I.S.T.A (13). The weight of 100 seeds were determined and measured in grammes in triplicates using electrical balance.

2.5 Germination Test

An absorbent material was placed inside Petri-dishes. Twenty (20), seeds of *Amaranthus hybridus* was introduced into a petri-dish lined with moist saturated absorbent material and placed in moist chamber at room temperature for ten days. The record of germination was taken at 5^{th} and 10^{th} day. Three replicate of each of the experiment was made. Germination rate was calculated according to the method of international rule for seed testing (13). The germination of the plumule was observed and recorded daily for germinating seed until all the available seed have germinated. Germination rate calculated according to the method of ISTA (13).

$$\text{Germination}\% = \frac{\text{Number of seed germination}}{\text{Number of seed on Petri} - \text{dish}} \times 100$$

2.6 Experimental Design

The experiments was conducted in the greenhouse and laboratory of department of Biological Sciences Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria during the 2022 farming season to investigate the effects of damping off fungus on seed germination of in *Amaranthus hybridus*. The treatment of the greenhouse experiment were arranged in Randomized Complete Block Design (RCBD) in triplicates and the laboratory experiments was conducted completely Randomized Design (CRD) with three replications. (14)

Twenty (20) *Amaranthus hybridus* seeds were plated in the five Petri-dishes each. Petri-dishes with content were moistened everyday with distilled water and incubation done at room temperature. The experiment was arranged in a Completely Randomized Design (CRD) with three replications. Germination count was conducted for seven days after which the physiological seed quality indices and attributes were determined: germination percentage (G%), germination index (GI), germination rate index (GRI) and seedling vigor index (I) as follows:

$$G\% = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

$$\text{GI} = = \text{Number of germinated seeds} + \text{Days of first count} + = \frac{\text{Number of germinasted seed}}{\text{days of final count}}$$

$$\text{GRI} = = \frac{\text{GI}}{G\%}$$

$$\text{I} = \text{Germination percentage} \times \text{Seedling shoot length (cm)} + \text{seedling root length (cm)} \ldots \ldots . (14)$$

2.7 Data analysis

Data obtained from the samples of tomato and okra landraces were transformed to percentages and subjected to analysis of variance (ANOVA) at $P \leq 0.05$ to test for the significant difference among the means and Duncan Multiple range test (DMRT) was used to separate the mean where there were differences.

3 Results

3.1 Identification of Seed borne fungus

The fungus *Rhizoctonia solani* was found associated with the seeds of *Amaranthus hybridus* causing damping off disease. The percentage of occurrence of the fungal isolates in the seeds was 75% from the infected farmland.

3.2 Effects of the physiological seed quality indices and attributes on the germination of *Amaranthus hybridus*

Mean squares for germination characteristics of *Amaranthus* infected and uninfected with *Rhizoctonia solani* a fungus responsible for damping-off diseases in *Amaranthus* was presented in Table 1. The result revealed that the treatment recorded a significant effect at ($p \leq 0.05$) on 100 seed weight and a highly significant effect ($p \leq 0.01$) on germination percentage, germination index, germination rate index and vigor index as presented in Table 1..

Table 1 Mean Squares for Germination Characteristics of Seeds of Amaranthus with the treatment

Sample	100 Seed weight (mg)	Germination %	Germination Index	Germination Rate Index	Vigor Index
Treatment	291.21*	123.45**	43.54**	6432.11**	9654.45**
Error	54.70	75.23	9.43	853.87	37813.12

KEY: ** = Significant at ($p \leq 0.01$); * = Significant at ($p \leq 0.05$)

Mean effects for the seed germination characteristics of *Amaranthus hybridus* infected and uninfected plants with *Rhizoctonia solani* causing damping-off diseases was presented in Table 2. The result indicated that seeds from uninfected plants recorded superior seed weight (8.97mg) as compared with the infected seeds (5.21mg). On the other hand, there was significantly higher germination percentage (98.67%) observed in *Rhizoctonia solani* uninfected seeds compared with seeds of *Amaranthus* plants that were infected with damping off diseases (32.13%). Germination index

was observed to be higher in seeds of uninfected plants (9.34) and lower in seeds of infected plants (4.34). Germination rate index and vigor index were also observed to follow a similar trend as presented in Table 2.

Table 2 Mean Effect for Germination Characteristics of Seeds of A*maranthus* Infected and Uninfected with *Rhizoctonia solani* (Damping-off Disease)

***Amaranthus* Seed**	**100 Seed weight (mg)**	**Germination %**	**Germination Index**	**Germination Rate Index**	**Vigor Index**
Rhizoctonia solani infected seeds	5.21b	32.13b	4.34b	231.11b	324.54b
Rhizoctonia solani uninfected seeds	8.97a	98.67a	9.34a	623.98a	798.33a

*Means with the same letter in a column are not significantly different

4 Discussion

The fungus *Rhizoctonia solani* that was isolated from the infected *Amaranthus hybridus* seeds in this study had been reported earlier by (8, 10, 17) to be fungal species associated with damping off disease both in the green house and field. This was further supported by the reports of (5, 22) that *Rhizoctonia* species are the most frequent fungal genera isolated from unsterilized *Amaranthus hybridus* varieties. The main reason for this observation might be because these fungus are primarily ubiquitous.

The mean weight obtained for *Amaranthus hybridus* infected seeds was (5.21mg) while that of uninfected seeds was (8.97mg) was reported in this study. The observed increase in the development of damping off disease in *Amaranthus* as the seed weights increased showed that these are pre-disposing factors to damping off disease. This was not in agreement with the reports of (3) who reported that seeds with small weight are pre-disposed to seedling disease. Mean weight of Infected seeds of *Amaranthus hybridus* were small and the development of damping off disease might be due to lateness in emergence which pre-disposes it to seed or soil borne light pathogens , this was reported by (6).

The highest germination percentage (98.67%) was obtained from uninfected *Amaranthus hybridus* seeds. This was in agreement with the reports of (19) who reported that untreated okra seeds germinate fast and was able to escape seed pathogens in the field. The low seed germination percentage of infected plants were susceptible to damping off disease as reported in this study. These observations was similar to the reports by (21), who reported that low germination percentage of seeds pre-disposes to susceptible infectious seedling disease. (16) also reported that seed-borne pathogens can promote damping-off which involves non-germination, prevention of seedling emergence after germination, or the rotting and collapse of seedlings at the soil level.

The Germination index, Germination rate index and vigor index was observed to be low in seeds of infected plants confirmed that the fungus *Rhizoctonia solani* is causative microbe responsible damping off disease of the plants . This was earlier been reported by (2), that many seed-borne pathogens become active when seeds are sown, which may result in seed decay or pre- or post- emergence damping off, thereby reducing desired plant population in the farmers' fields. Seed-borne pathogens, particularly, fungi induce qualitative changes in the physio-chemical properties of seeds such as protein content (2) that are fundamental in the germination process. (8, 18) also reported that a collective of fungal pathogens contaminants, reduced seed vigor and inhibited seed germination and seedling growth.

The significant effects observed of the fungus in this study on both seed and seedlings are highly virulence on the infected plants. This may explain their strong relationship with the seeds and ability to be susceptible to damping off disease in the field. Such developments influence the ultimate crop yield and reduce the commercial value of the crop as well as reported by (8, 20)

5 Conclusion

The research revealed that a very good association between the seed borne fungus *Rhizoctonia solani* causing damping off disease and seed germination failure of *Amaranthus hybridus* seeds was investigated. Therefore, research on other vegetable seed crops from the different agricultural zones of the state should be undertaken in order to identify the exact pathogens regarding the prevalence of seed borne fungus and the role they do play on seed weight, seed germination, germination rate index and vigor index.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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