



**Full Length Article**

# Effects of Seed-borne Fungi on Germination and Seedling Growth of Watermelon (*Citrullus lanatus*)

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## ABSTRACT

The effect of *Mucor racemosus* and *Rhizopus nigricans* inocula (1 & 0.1 g L<sup>-1</sup> distilled water) and a seed dressing fungicide Seedplus® (1.25 g 500 g<sup>-1</sup> seeds) on germination and seedling growth of watermelon (cv. Chaliston gray) was investigated. It was confirmed that the combined inocula with higher density caused significantly poor germination and seedling growth ( $P \leq 0.05$ ) than those seeds inoculated with lower inocula density. Higher germination percentage and more vigorous seedling growth were observed with single fungus inoculation than with the two fungi inoculation. Dressing of the fungi-infected seeds with Seedplus® 30 WS significantly improved germination percentage and seedling vigour of watermelon ( $P \leq 0.05$ ) but not as high as that of the control.

**Key Words:** *Mucor racemosus*; *Rhizopus nigricans*; Germination percentage; Seed plus; Watermelon

## INTRODUCTION

In Nigeria, the largest production of watermelon (*Citrullus lanatus* Thumb Family: Cucurbitaceae) comes from the northern part of the country, where the suitable agro-ecology is found (IITA, 2007). Watermelon is cultivated extensively for its pleasant-tasting fruit (Mendel *et al.*, 2005). Its fruits are mostly threatened by some pathogenic fungi, which are seed-borne and also soil-borne such as *Mucor* spp. and *Rhizopus* spp. (Pamela & Tom, 2006). The effects of such fungi on the seedlings growth include poor germination and less vigorous seedlings. These usually result in low yield and low income arising from poor yield quantity and quality.

Although much work has been done on investigating and discovery of fungal diseases that affect the fruits, there are no substantial report on the effect of pathogenic fungi on the seeds and seedling of watermelon. *R. stolonifer* a type of black mould has a wide host range and can affect over 300 plant species including fruits, vegetables and ornamentals (Farr *et al.*, 2007). *Mucor* spp., which is a soil-borne pathogen may infect the fruit and stem of several plants such as pears and apple (Michailides & Spotts, 1990). This study assessed the effect of *Mucor racemosus* and *Rhizopus nigricans* individually and in combination and confirmed the efficacy of Seedplus® fungicide on germination and seedling of infected watermelon seeds.

## MATERIALS AND METHODS

**Collection of seed sample.** Watermelon seeds (cv Chaliston

gray commonly grown in Nigeria) were obtained from Gwadabe market, Minna, Nigeria (Lat. 4° 30' N of the Equator & Long. 10° 30' E), where most farmers procure their planting seeds.

**Inoculum production and identification.** Confirmed strains of *R. nigricans* and *M. racemosus* by CABI Biosciences Identification Services (IMI 392668 & 392668) were maintained on cowpea seeds kept in Biochemistry Laboratory of Federal University of Technology (FUT), Minna, Nigeria. The infected seeds samples were aseptically placed in 90 mm diameter Petri dishes containing 15 mL each of an autoclaved Potato Dextrose Agar, (PDA, Oxoid) added with 0.05 g L<sup>-1</sup> chloramphenicol. It was incubated in lamina hood at 28°C and examined from 2-3 days in order to obtain the pure culture of the inoculum. On the third day of incubation, hyphal fragments inoculum was prepared by flooding the surface of the agar slant with sterile distilled water and gently scraping the surface of the sporing surface with a loop. Hyphal structures were germ tubes and were at least five times as long as the diameter of the spores. The resulting suspension with spores was then filtered through sterile gauze. To reconfirm the identity of the fungi, mycelia speck from each colony were aseptically placed on a slide, stained with lactophenol blue, covered with slips and viewed under microscope (40 X). The identification was accomplished using fungi catalogue in the Microbiology Department, FUT, Minna, Nigeria.

**Inoculum quantification and inoculation.** The hyphal strands of each fungus were diluted by adding sterile distilled water 1 and 0.1 g L<sup>-1</sup> water to obtain the working

suspensions. One hundred seeds (5 g) were pre-inoculated with 2 mL of each of the hyphal suspension concentrations by soaking for 20 min i.e., 0.4 L kg<sup>-1</sup> seeds. The treatments included seeds pre-inoculated with high and low density of *M. racemosus* and *R. nigricans* (g L<sup>-1</sup>) and 0.1g L<sup>-1</sup> distilled water as follows:

*M. racemosus* (g L<sup>-1</sup>) + *R. nigricans* (g L<sup>-1</sup>); *M. racemosus* (0.1 g L<sup>-1</sup>) + *R. nigricans* (0.1 g L<sup>-1</sup>); *M. racemosus* (g L<sup>-1</sup>), *R. nigricans* (g L<sup>-1</sup>); *M. racemosus* (0.1 g L<sup>-1</sup>); *R. nigricans* (0.1 g L<sup>-1</sup>) and *M. racemosus* (g L<sup>-1</sup>) + *R. nigricans* (g L<sup>-1</sup>) plus (10% imidacloprid + 10% metalaxyl + 10% carbendazim 2.5 g Kg<sup>-1</sup> seeds (Seedplus® 30 WS, Jiangsu Flag Industry Co., Ltd, Nianjing, China) and the control with un-inoculated seeds.

A completely randomized experimental design with three replicates was involved. Ten seeds each were placed in 24 Petri dishes containing three layers of blotters moistened with 10 mL distilled water, set to germinate at 28±2°C in the incubator and observed for eleven days for germination, fungal infection and seedling performance. Germination percentage, length of radicle and length of plumule were recorded daily as from fifth to 11<sup>th</sup> days after sowing (DAS). Seedling performance index was calculated at 8 DAS according to Randahawa *et al.* (1985) and modified as follows:

$$VI = (PL + RL) \times GP$$

Where VI = Vigor index; PL = plumule length (cm); RL = radicle length (cm); GP = germination percentage (%).

Data collected were subjected to analysis of variance (ANOVA) and means were separated with Duncan Multiple Range Test (DMRT) by using SAS (1997) Statistical Package and *P* values < 0.05 were considered statistically significant.

## RESULTS

Five days after sowing (DAS), the germination percentage of watermelon seeds inoculated with high inoculum density (*M. racemosus* + *R. nigricans* (g L<sup>-1</sup>)) had the lowest percentage germination (46.67%). The highest germination percentage was observed in the control (88.33%) and this was significantly different (*P* ≤ 0.05) from the seeds applied with *M. racemosus* + *R. nigricans* (g L<sup>-1</sup> distilled water) At 6, 7 and 8 DAS, germination percentage of seeds applied with *M. racemosus* + *R. nigricans* (g L<sup>-1</sup> or 0.1g L<sup>-1</sup> distilled water) was significantly lower (*P* ≤ 0.05) than that of other treatments (Table I).

As evident from Table II, at 5 DAS, length of plumule (2.45 cm) from the seeds applied with *M. racemosus* + *R. nigricans* (g L<sup>-1</sup>) was significantly lower (*P* ≤ 0.05) than those applied with *M. racemosus* + *R. nigricans* (0.1 g L<sup>-1</sup>). The plumule length of seeds in the control was the highest (6.75 cm) but was only significantly higher (*P* ≤ 0.05) than for seeds applied with *M. racemosus* + *R. nigricans* (g L<sup>-1</sup>).

At 6 DAS, seeds applied with *M. racemosus* + *R. nigricans* (g L<sup>-1</sup>) had the least plumule length (3.55 cm) and this was significantly lower (*P* ≤ 0.05) than for all other treatments except those applied with *M. racemosus* + *R. nigricans* (0.1 g L<sup>-1</sup>). At 7 and 8 DAS, seeds in the control had the highest plumule length (11.50 cm) and (12.50 cm), respectively. This was significantly higher (*P* ≤ 0.05) than for seeds applied with *M. racemosus* + *R. nigricans* (g L<sup>-1</sup>).

At 5 DAS, radicle growth was highest in the control but not significantly different (*P* ≤ 0.05) from fungi inoculated seeds applied with Seedplus®. This was significantly higher (*P* ≤ 0.05) than for seeds applied with *M. racemosus* + *R. nigricans* (g L<sup>-1</sup>) and *R. nigricans* only (g L<sup>-1</sup>). At 6 and 7 DAS, radicle length of seeds applied with *M. racemosus* + *R. nigricans* (g L<sup>-1</sup>) was significantly lower (*P* ≤ 0.05) than for those applied with *M. racemosus* only (0.1 or g L<sup>-1</sup>), Seedplus® and the control. At 8 DAS, seeds applied with *M. racemosus* + *R. nigricans* (g L<sup>-1</sup>) had the least radicle length (4.83 cm) and was significantly lower (*P* ≤ 0.05) than those applied with *M. racemosus* (0.1 g L<sup>-1</sup>), Seedplus and the control. Seedling vigour index was significantly lower (*P* ≤ 0.05) in seeds applied with *M. racemosus* + *R. nigricans* (g L<sup>-1</sup>) than for those applied with lower inoculum density (0.1 g L<sup>-1</sup>). Highest vigour index was obtained in the control (1.99) and this was not significantly different (*P* ≤ 0.05) from the inoculated seeds with the fungi but with Seedplus® treatment (Table III).

## DISCUSSION

Watermelon seeds inoculated with the fungi without fungicide treatment in this study exhibited some pathogenic symptoms such as root rot. Moss and Smith (2006) earlier reported that pathogenic seed-borne fungi include *R. nigricans*, *Mucor* spp. and *Fusarium oxysporum*. Mehrotra and Aggarwal (2003) reported that such fungi could seriously retard seed germination through softening and necrosis of tissues. They also confirmed the association of seed-borne fungi with seed viability, wilting of plants and stem flaccidity. Incidences of *R. nigricans* and several other pathogenic seed-borne fungi on seeds have been reported by Leslie *et al.* (2005) and Anjorin *et al.* (2008). The factors influencing the development of seed-borne fungi include the moisture content of the seed, prevailing temperature, storage period and degree of seed invasion with the pathogen. Others are level of host genetic resistance, activities of insects and mites and amount of foreign materials in the seed lot (Miller & Trenholen, 1994).

The inhibition of radicle and plumule growth especially by seeds applied with high inoculum density led to lower germination percentage of up to 50% (Lin & Ehret, 1991; Gilbert & Tekauz, 1995; Menzies *et al.*, 1996). Pathogenic fungi may only be present at such low density such that their inoculum potential is low. Thus infected plants may not show significant symptoms of infection even though the pathogen is present in their cell or tissue

**Table I. Effects of inoculum density and types of pathogenic mould on germination percentage of watermelon**

Treatment	Germination percentage			
	5 DAS (%)	6 DAS (%)	7 DAS (%)	8 DAS (%)
<i>M. racemosus</i> + <i>R. nigricans</i> (g L <sup>-1</sup> )	46.67b*	46.67c	46.67c	46.67c
<i>M. racemosus</i> + <i>R. nigricans</i> (0.1g L <sup>-1</sup> )	60.00ab	60.00bc	60.00bc	60.00bc
<i>M. racemosus</i> (g L <sup>-1</sup> )	86.67a	93.33a	93.33a	93.33a
<i>R. nigricans</i> (g L <sup>-1</sup> )	73.33ab	73.33abc	73.33abc	73.33ab
<i>Racemosus</i> (0.1g L <sup>-1</sup> )	86.67a	93.33a	93.33a	93.33a
<i>R. nigricans</i> (0.1g L <sup>-1</sup> )	86.67a	93.33a	93.33a	93.33a
<i>M. racemosus</i> + <i>R. nigricans</i> (g L <sup>-1</sup> ) + Seedplus®	78.00ab	80.00ab	80.00ab	80.00ab
Control	88.33a	93.33a	93.33a	93.33a

\*Within each column, means followed by the same letter(s) are not significantly different ( $P \leq 0.05$ ) according to Duncan Test. \*\*DAS – Days after sowing

**Table II. Effects of inoculum density and types of pathogenic mould on plumule length of watermelon seeds**

Treatment	Plumule length (cm)			
	5 DAS**	6 DAS	7 DAS	8 DAS
<i>M. racemosus</i> + <i>R. nigricans</i> (g L <sup>-1</sup> )	3.45b*	3.55b	4.27b	4.50c
<i>M. racemosus</i> + <i>R. nigricans</i> (0.1 g L <sup>-1</sup> )	4.78ab	4.88ab	7.30a	7.86bc
<i>M. racemosus</i> (g L <sup>-1</sup> )	6.48a	6.48a	9.07a	11.26ab
<i>R. nigricans</i> (g L <sup>-1</sup> )	5.72a	5.72a	8.40a	9.50ab
<i>M. racemosus</i> (0.1 g L <sup>-1</sup> )	6.60a	6.70a	10.03a	11.10ab
<i>R. nigricans</i> (0.1 g L <sup>-1</sup> )	5.45a	5.45a	7.77a	10.90ab
<i>M. racemosus</i> + <i>R. nigricans</i> (g L <sup>-1</sup> ) + Seedplus®	5.33a	5.33a	10.97a	11.33ab
Control	6.75a	8.95a	11.50a	12.50a

\*Within each column, means followed by the same letter(s) are not significantly different ( $P \leq 0.05$ ) according to Duncan Test. \*\*DAS – Days after sowing

**Table III. Effects of inoculum density and types of pathogenic mould on radicle length vigor indices of watermelon seeds**

Treatment	Radicle length (cm)				V.I*** (x 100)
	5 DAS**	6 DAS	7 DAS	8 DAS	8 DAS
<i>M. racemosus</i> + <i>R. nigricans</i> (g L <sup>-1</sup> )	3.17a*	4.47 <sup>a</sup>	4.57 <sup>a</sup>	4.83 <sup>a</sup>	0.39 <sup>d</sup>
<i>M. racemosus</i> + <i>R. nigricans</i> (0.1 g L <sup>-1</sup> )	4.40 <sup>a</sup>	5.90 <sup>ab</sup>	6.37 <sup>ab</sup>	6.87 <sup>ab</sup>	0.89 <sup>c</sup>
<i>M. racemosus</i> (g L <sup>-1</sup> )	5.25 <sup>b</sup>	7.00 <sup>b</sup>	7.40 <sup>b</sup>	7.93 <sup>ab</sup>	1.53 <sup>ab</sup>
<i>R. nigricans</i> (g L <sup>-1</sup> )	4.50 <sup>a</sup>	6.38 <sup>b</sup>	6.47 <sup>ab</sup>	7.23 <sup>a</sup>	1.84 <sup>a</sup>
<i>M. racemosus</i> (0.1g L <sup>-1</sup> )	5.67 <sup>b</sup>	7.48 <sup>b</sup>	7.96 <sup>b</sup>	8.70 <sup>b</sup>	1.21 <sup>bc</sup>
<i>R. nigricans</i> (0.1g L <sup>-1</sup> )	6.37 <sup>b</sup>	5.48 <sup>ab</sup>	6.53 <sup>ab</sup>	7.20 <sup>ab</sup>	1.68 <sup>ab</sup>
<i>M. racemosus</i> + <i>R. nigricans</i> (g L <sup>-1</sup> ) + Seedplus®	5.38 <sup>b</sup>	7.38 <sup>b</sup>	8.30 <sup>b</sup>	8.68 <sup>b</sup>	1.60 <sup>ab</sup>
Control	6.38 <sup>b</sup>	7.10 <sup>b</sup>	8.06 <sup>b</sup>	8.70 <sup>b</sup>	1.99 <sup>a</sup>

\*Within each column, means followed by the same letter (s) are not significantly different ( $P \leq 0.05$ ) according to Duncan Test. \*\*DAS – Days after sowing \*\*\* V.I. = vigor index

(Sanogo & Moorman, 1993). Higher inoculum density can overcome the plant's defense mechanisms and cause death. However the actual level of inoculum needed to overcome the host's defenses would vary with the environmental conditions (Paternotte, 1992). Whether this is because of an increased severity of infection caused by increased number of primary infections at few sites on the roots, or an increased number of infection sites along the roots, is not known. It may be that the seeds applied with low inoculum density are able to overcome the low level of infections and once the defence responses are activated the plants are able to tolerate the infections that occurred from secondary inoculum produced from primary infections (Menzies *et al.*, 1996). A strong linear relationship between pathogen inoculum density and growth and yield parameters monitored by Sanogo and Moorman (1993) are good indications of how the increasing density of the pathogen inoculum increases the stress on the host plants.

The relatively higher radicle growth observed on the fungal inoculated seeds applied with imidacloprid, metalaxyl and carbendazim (Seedplus®) and also in the control was in line with the report of Paternotte (1992) on disease development of *Pythium* in glass house cucumber. Seeds treated with Seedplus® recorded relatively high percentage germination. This could be due to the systemic action of Seedplus®, which is strongly inhibitory to hyphal development and fungal spore germination (Maynard & Hopkins, 1999). This supports the fact that this seed dressing fungicide was effective in the control of the fungi infection (Freshpatents.com, 2005).

## CONCLUSION

This study confirmed that *M. racemosus* and *R. nigricans* were capable of causing considerable poor germination and seedling in watermelon. It was also

confirmed that the higher the inoculum density especially when the fungi are combined, the greater the pathogenic capacity on watermelon seeds. Thus infected seeds with low inoculum density had relatively better germination percentage, plumule and radicle length and seedling than those applied with high inoculum density. Better germination and seedling vigour was observed when only one fungus was involved than when two fungi were involved. Hence inoculum level on seeds should be reduced to the tolerable level through the use of seed dressing fungicide such as imidacloprid, metalaxyl and carbendazim (Seedplus®) before sowing. This could greatly improve radicle and plumule development, germination and vigour indices of watermelon seeds. Farmers should preferably sow watermelon seeds that is clean, fungicide-dressed or fungal-resistant.

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