academicJournals

Vol. 8(5), pp. 232-236, May 2014
DOI: 10.5897/AJPS08.090
Article Number: 6221E9844931
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Effect of seed-borne fungi on germination and seedling vigour of watermelon (*Citrullus lanatus* thumb)

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Received 5 November, 2008; Accepted 5 February, 2014

The effect of *Mucor racemosus* and *Rhizopus nigricans* inoculation (g and 0.1 g L^{-1} distilled water) and a seed dressing fungicide Seedplus® (1.25 g 0.5 kg^{-1} seeds) on germination and seedling vigour of watermelon (cv. Chaliston gray) was investigated. The experiment involved a complete randomized design (CRD) with three replicates. It was confirmed that the combined inocula with higher density caused significantly poor germination and low seedling vigour (P ≤ 0.05) than those seeds inoculated with lower inocula density. Higher germination percentage and seedling vigour 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 ≤ 0.05) but not as high as that of the control.

Key words: *Mucor racemosus*, *Rhizopus nigricans,* Seedplus.

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 nature. 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 and Tom, 2006). The effects of such fungi on the seedlings include poor germination, low seedling vigour and even complete failure of 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. *Rhizopus 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, and 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.

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Table 1. Effects of inoculum density and types of pathogenic mould on germination percentage of watermelon.

Treatment	Germination percentage			
	5DAS (%)	6DAS (%)	7DAS (%)	8DAS (%)
M. racemosus + *R. nigricans* (g L^{-1})	46.67^{b}*	46.67^{c}	46.67^{c}	46. 67^{c}
M. racemosus + *R. nigricans* (0.1gL^{-1})	60.00^{ab}	60.00^{bc}	60.00^{bc}	60. 00^{bc}
M. racemosus (g L^{-1})	86.67^{a}	93.33^{a}	93.33^{a}	93.33^{a}
R. nigricans (g L^{-1})	73.33^{ab}	73.33^{abc}	73.33^{abc}	73.33^{ab}
M. racemosus (0.1 g L^{-1})	86.67^{a}	93.33^{a}	93.33^{a}	93.33^{a}
R. nigricans (0.1 g L^{-1})	86.67^{a}	93.33^{a}	93.33^{a}	93.33^{a}
M. racemosus + *R. nigricans* (g L^{-1}) + Seedplus®	78.00^{ab}	80.00^{ab}	80.00^{ab}	80.00^{ab}
Control	88.33^{a}	93.33^{a}	93.33^{a}	93.33^{a}

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

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^{1} N of the Equator and Long. 10° 30^{1} 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. 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^{-1} 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 after 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 which are germ tubes were at least five times as long as the diameter of the spores. The resulting suspension with spores was then filtered off 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 (40x). The identification was accomplished using fungi catalogue in the Microbiology Department in F.U.T, Minna.

Inoculum quantification and inoculation

The hyphal strands of each fungus were diluted by adding sterile distilled water 1 g and 0.1g l^{-1} 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, that is, 0.4 L kg^{-1} seeds. The treatments included seeds pre-inoculated with high and low density of *M. racemosus* and *R. nigricans* (g 1 L^{-1}) and 0.1g L^{-1} distilled water as follows:

M. racemosus (g L^{-1}) + *R. nigricans* g L^{-1}; *M. racemosus* (0.1 g L^{-1}) + *R. nigricans* (0.1 g L^{-1}) *M. racemosus* (g L^{-1}), *R. nigricans* (g L^{-1}), *M. racemosus* (0.1 g L^{-1}), *R. nigricans* (0.1 g L^{-1}) and *M. racemosus* g L^{-1}+ *R. nigricans* (g L^{-1}) plus (10% imidacloprid + 10% metalaxyl + 10% carbendazim 2.5 g kg^{-1} seeds (Seedplus® 30 WS, Jiangsu Flag Industry Co., Ltd, Nianjing, China) and the control with uninoculated 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 vigour.

Germination percentage, length of root and length of plumule were recorded daily as from the fifth to eleventh day. Germination percentage, length of radicle and length of plumule were recorded daily as from 5 to 11 days after sowing (DAS). Vigour index was calculated at 8 DAS according to Randahawa et al. (1985) and modified as follows:

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

Where VI = Vigour 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) and *P* values <0.05 were considered statistically significant.

RESULTS

At five days after sowing (DAS), the germination percentage of watermelon seeds inoculated with high inoculum density (*M. racemosus* + *R. nigricans* (g L^{-1}) had the lowest percentage germination (46.67%) (Table 1).

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* (gL^{-1}). At 6, 7 and 8 DAS, germination percentage of seeds applied with *M. racemosus* + *R. nigricans* (g L^{-1} or 0.1gL^{-1}) was significantly lower (P≤ 0.05) than that of other treatments (Table 2).

At 5 DAS, length of plumule (2.45 cm) from the seeds applied with *M. racemosus* + *R. nigricans* (g L^{-1}) was significantly lower (P≤ 0.05) than those applied with *M. racemosus* + *R. nigricans* (0.1g L^{-1}). The plumule length

Table 2. Effect of inoculum density and types of pathogenic mould on plumule length of watermelon seeds.

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

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

Table 3. Effects of inoculum density and types of pathogenic mould on radicle length vigour indices of watermelon seeds.

Treatment	Radicle length (cm)				V.I*** (x100)
	5 DAS**	6 DAS	7 DAS	8DAS	8DAS
M. racemosus + *R. nigricans* (gL^{-1})	3.17^{a*}	4.47^{a}	4.57^{a}	4.83^{a}	0.39^{d}
M. racemosus + *R. nigricans* (0.1 gL^{-1})	4.40^{a}	5.90^{ab}	6.37^{ab}	6.87^{ab}	0.89^{c}
M. racemosus (g L^{-1})	5.25^{b}	7.00^{b}	7.40^{b}	7.93^{ab}	1.53^{ab}
R. nigricans (g L^{-1})	4.50^{a}	6.38^{b}	6.47^{ab}	7.23^{a}	1.84^{a}
M. racemosus (0.1 g L^{-1})	5.67^{b}	7.48^{b}	7.96^{b}	8.70^{b}	1.21^{bc}
R. nigricans (0.1 g L^{-1})	6.37^{b}	5.48^{ab}	6.53^{ab}	$7.20^{a\ b}$	1.68^{ab}
M. racemosus + *R. nigricans*(g L^{-1})+ Seedplus®	5.38^{b}	7.38^{b}	8.30^{b}	8.68^{b}	1.60^{ab}
Control	6.38^{b}	7.10^{b}	8.06^{b}	8.70^{b}	1.99^{a}

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

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^{-1}). At 6 DAS, seeds applied with *M. racemosus* + *R. nigricans* (g L^{-1}) 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* (0.1g L^{-1}). At 7 and 8 DAS, seeds in the control had the highest plumule length (11.50 and 12.50 cm, respectively). This was significantly higher (P≤ 0.05) than for seeds applied with *M. racemosus* + *R. nigricans* (g L^{-1}) (Table 3).

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^{-1}) and *R. nigricans* only (g L^{-1}). At 6 and 7 DAS, radicle length of seeds applied with *M. racemosus* + *R. nigrican s* (g L^{-1}) was significantly lower (P≤ 0.05) than for those applied with *M. racemosus* only (0.1 or g L^{-1}), Seedplus® and the control. At 8 DAS, seeds applied with *M. racemosus* + *R. nigricans* (g L^{-1}) 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^{-1}), Seedplus and the control. Seedling vigour index was significantly lower (P≤ 0.05) in seeds applied with *M. racemosus* + *R. nigricans* (g L^{-1}) than for those applied with lower inoculum density (0.1 g L^{-1}). 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.

DISCUSSION

Water melon 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 and 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% (Linn and Ehret, 1991; Gilbert and Tekauz, 1995; Menzies et al., 1996). Pathogenenic 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 (Sanogo and 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 submission 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 and 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 confirms that *M. racemosus* and *R. nigricans* were capable of causing considerable low germination and seedling vigour 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 higher germination percentage, plumule and radicle length and seedling vigour 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 are clean, fungicide-dressed or fungal-resistant.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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