African Journal of Agricultural Research Vol. 6(12), pp. 2655-2659, 18 June, 2011
Available online at http://www.academicjournals.org/AJAR
DOI: 08.5897/AJAR09.480
ISSN 1991-637X ©2011 Academic Journals

Full Length Research Paper

Germinability and seedling vigour of physic nut (*Jatropha curcas* L.) seeds inoculated with seed-borne fungi

Samuel Toba Anjorin[1]*, Olushola Ruth Omolewa[1] and Ezekiel Adebayo Salako[2]

[1]Department of Crop Production, Federal University of Technology, Minna, Nigeria.
[2]Department of Crop Science, University of Abuja, Abuja, Nigeria.

Accepted 7 February, 2011

Germinability and seedling vigour of physic nut (*Jatropha curcas* L.) seeds inoculated with *Aspergillus flavus* and *Rhizopus nigricans* were investigated in a germination chamber and in plastic pots in a screen house at Federal University of Technology (FUT), Minna. The seeds were pre-inoculated with the fungi for 72 h and 0 h before sowing and compared with those applied with Seedplus® 30WS fungicide. The experiment which involved completely randomised design had eight treatments in three replicates. It was confirmed in the germination chamber that seeds inoculated with *A. flavus* + *R. nigricans* for 72 h before sowing had the lowest percentage germination (20%) at 8 DAS while the uninoculated seeds (control) had the highest (87%). In the pot at 20 DAS, seeds applied with combined *A. flavus* and *R. nigricans* for 72 h before sowing had the least seedling vigour index (0.38) while the uninoculated control had the highest vigour index (1.89). Though the presence of *A. flavus* + *R. nigricans* on physic nut seeds adversely affected germination and seedling vigour, seed-dressing with Seedplus® significantly reduced the pathogenic effect of the fungi. There is need for confirmation of this experiment under field condition in different agroecological conditions.

Key words: Germinability, physic nut, seedling vigour, *Aspergillus flavus*, *Rhizopus nigricans*, Seedplus®.

INTRODUCTION

Physic nut, a deciduous monoecious shrub of up to 5 - 8 m tall, is widely cultivated in the tropics as a living fence in fields and settlements as it is not usually browsed by grazing animals (Duke and Atchley, 1985). The plant can be grown in areas with low rainfall and non-croppable sites (Heller, 1996). Preparations of all parts of the plant including seeds, leaves and bark, fresh or as a decoction are used in traditional medicine and veterinary purposes.

The clear oil pressed from the seeds is used for illumination and lubrication (Gaydou et al., 1982). The latex is strongly inhibitory to watermelon mosaic virus (Tewari and Shukla, 1982). The bark is used as a rat and fish poison (Watt and Breyer-brandwiljk, 1982). Its considerable potential as an oil crop for bio-fuel purposes at relatively low costs and modest demands on the local agro-ecosystem has received much attention in recent years. Jatropha latex is reported to be effective against fungal pathogen. Also extracts from crushed seeds showed some fungicidal properties (Liu et al., 1996). Some seed-borne fungi were found in association with *Jatropha curcas* seedlings since there is low or no latex formed at seedling stage (Garcia and Lawas, 1990). There are scanty reports on the effect of seed-borne pathogenic fungi on *J. curcas* seeds and seedlings. Thus this study assessed the germinability and seedling vigour of physic nut seeds pre-inoculated with *Aspergillus flavus* and *Rhizopus nigricans* compared with those applied with Seedplus® fungicide.

*Corresponding author. E-mail: oyindamola35@yahoo.com

MATERIALS AND METHODS

Collection of seeds sample, preparation of culture medium and inocula preparation

Physic nut seeds were obtained from Gwagwalada, Abuja, Nigeria in January, 2008. They were disinfected with sodium hypochlorate and air-dried on the Laboratory benches. Potato Dextrose Agar (PDA) was prepared in the Laboratory of Crop production

Table 1. Effects of *A. flavus* and *R. nigricans* and the duration of preinoculation on physic nut seeds on percentage germination.

Treatment	Percentage germination day after sowing			
	5	6	7	8
A. flavus (72 h)	40.00^{bc}	46.70^{bc}	53.30^{cb}	54.40^{bcd}
A. flavus (0 h)	60.00^{ab}	63.60^{ab}	66.70^{ab}	67.00^{abc}
R. nigricans (72 h)	26.70^{c}	33.30^{cd}	40.00^{cd}	41.00^{cde}
R. nigricans (0 h)	33.30^{bc}	33.30^{cd}	33.30^{cd}	43.30^{de}
A. flavus+ *R. nigricans* (72 h)	20.00^{c}	20.00^{d}	20.00^{d}	20.00^{e}
A .flavus +*R. nigricans* (0 h)	33.30^{bc}	40.00^{cd}	40.00^{cd}	41.00^{cde}
A.flavus +*R. nigricans* +Seed plus®	46.70^{ab}	66.30^{cb}	73.30^{ab}	73.40^{ab}
Control	73.33^{a}	80.00^{a}	86.70^{a}	87.70^{a}

Column means followed by common letter(s) are not significantly different (P > 0.05) by DMRT.

Department in FUT, Minna. The prepared medium (39 g/L distilled water) was sterilized using autoclave at 121°C for 15 min, and 50 mg/ml of chloramphenicol was added to the sterile medium to suppress the growth of bacteria and was further stirred properly and allowed to cool. Fifteen milliliters PDA was poured into each of the 12 Petri dishes and was allowed to gel.

The duly identified fungi isolates reserved in the Microbiology Laboratory of FUT, Minna were introduced into the PDA in the Petri dishes using a sterile inoculating needle. The plates were labeled and incubated at a temperature of 28°C + 1 for 7 days in the Laboratory. Confirmatory identification of the fungi species was through the macroscopic features and by viewing stained samples under microscope and characteristics compared with fungi identification catalogue in Microbiology Laboratory FUT, Minna.

Inoculation of physic nut seeds with *Aspergillus flavus, Rhizopus nigricans* and Seedplus® before sowing

Ten grams each of *A. flavus* and *R. nigricans* mycelia was added to 20 ml of distilled water and were stirred thoroughly singly and in combination. 60 seeds were soaked for two minutes in the diluted *A. flavus* and *R. nigricans* mycelia in a beaker. Three of the inoculated seeds were allowed to stand for 72 h before sowing while three others were sown immediately after inoculation (0 h). Other treatments were the preinoculated seeds for 72 h dressed with fungicidal imidacloprid, metalaxyl and carbendazim - Seedplus® (2.5 g/Kg seeds) and the uninoculated control. Each set of seeds were sown in Petri dishes lined with moist filter paper in a germination chamber, and in plastic pots filled with sterilized soil. Garden soil sample collected from FUT main campus Gidan-kwanu, Minna, was sterilized at 70°C for 50 min (Russel, 1997), cooled and wetted accordingly. The experiment involved complete randomized design (CRD) with three replicates.

Data collection and statistical analysis

Data were collected on percentage germination, percentage emergence, length of radicle and plumule and seedling vigour in the germination chamber and in the pot. Vigour index was computed according to modified Randahawa et al. (1985) as follows:
In the germination chamber:

Vigour Index = PG (PL +RL)

Where, PG is the percentage germination; PL is the plumule length and RL is the radicle length.

In the pot:

Vigour Index = SE (SL + RL)

Where, SE is the seedling emergence; SL is the shoot length and RL is the root length.

The data were subjected to Analysis of Variance (ANOVA) and treatment means separated by Duncan Multiple Range Test (DRMT) at 5% level of probability using SAS (1997) Statistical Package.

RESULTS

Germination chamber experiment

The result of the effects of duration of preinoculation of the physic nut seeds with *A. flavus* and *R. nigricans* before sowing on percentage germination, length of radicle, plumule and seedling vigour in the germination chamber is presented in Tables 1 to 3. At 5 DAS, it was indicated that *R. nigricans* adversely affected germination than the *A. flavus*. Seeds inoculated with *A. flavus* + *R. nigricans* for 72 h had the lowest percentage germination. At 6 DAS, there was no significant difference (P>0.05) in the percentage germination from the seeds in the control, seeds applied with *A. flavus* for 0 h and those dressed with Seedplus®. At 7 and 8 DAS, there was also no significant difference (P>0.05) in the percentage germination of pre inoculated seeds dressed with Seedplus® and the control.

All through 5 to 8 DAS, the highest plumule length was observed in the seeds applied with Seedplus® while those applied with *A. flavus* + *R. nigricans* for 72 h had the least length of plumule (Table 2). At 7 DAS the seeds applied with Seedplus® showed a relatively high length of plumule but was significantly lower (P<0.05) than the control. At 8 DAS, length of plumule of seeds preinoculated with *A. flavus* + *R. nigicans* for 72 h was significantly lower (P≤0.05) than from other treatments. At 5 DAS the length

Table 2. Effects of *A. flavus and R. nigricans* and the duration of preinoculation on physic nut seeds on length of plumule.

Treatment	Length of plumule (cm) days after sowing			
	5	6	7	8
A. flavus (72 h)	2.30^{bc}	4.33^{ab}	4.70^{a}	5.00^{a}
A. flavus (0 h)	2.80^{abc}	5.20^{ab}	5.60^{ab}	6.00^{a}
R. nigricans (72 h)	3.50^{ab}	5.30^{ab}	5.50^{a}	5.50^{a}
R. nigricans (0 h)	4.25^{ab}	5.30^{ab}	6.10^{a}	6.40^{a}
A. flavus+ *R. nigricans* (72 h)	1.10^{c}	2.50^{b}	2.95^{a}	3.40^{b}
A .flavus +*R. nigricans* (0 h)	2.70^{abc}	3.90^{b}	4.40^{ac}	4.50^{a}
A.flavus +*R. nigricans* +Seed plus®	4.90^{a}	6.20^{ab}	6.60^{b}	6.80^{a}
Control	4.10^{ab}	6.30^{a}	6.70^{a}	7.00^{a}

Column means followed by common letter(s) are not significantly different (P>0.05) by DMRT.

Table 3. Effects of *A. flavus and R. nigricans* and the duration of preinoculation on physic nut seeds on length of radicle and seedling vigour.

Treatment	Length of radicle (cm) day after sowing				SVI
	5	6	7	8	(x100)
A. flavus (72 h)	2.60^{bc}	1.97^{b}	3.10^{ab}	3.00^{a}	0.45^{abc}
A. flavus (0 h)	5.60^{a}	4.00^{ab}	4.20^{ab}	4.10^{a}	0.67^{ab}
R. nigricans (72 h)	4.10^{abc}	3.70^{ab}	3.80^{ab}	3.70^{a}	0.37^{abc}
R. nigricans (0 h)	4.40^{ab}	3.20^{ab}	3.50^{ab}	3.60^{a}	0.32^{bc}
A. flavus+ *R. nigricans* (72 h)	1.80^{c}	2.60^{b}	2.65^{b}	2.60^{b}	0.12^{c}
A. flavus+*R. nigricans* (*0* h)	4.30^{ab}	3.20^{ab}	3.40^{ab}	3.30^{a}	0.29^{bc}
A. flavus + *R. nigricans* + Seedplus®	4.50^{ab}	3.80^{ab}	4.20^{ab}	4.60^{a}	0.75^{a}
Control	4.30^{ab}	5.20^{a}	5.60^{a}	5.65^{a}	0.83^{ab}

Column means followed by common letter(s) are not significantly different (P > 0.05) by DMRT. *SVI = seedling vigour index.

of radicle of preinoculated seeds applied with Seedplus® was significantly higher (P<0.05) than those applied with *A. flavus* + *R. nigricans* for 72 h. At 7 DAS, seeds applied with *A. flavus* + *R. nigricans* for 72 h produced the least radicle length (2.65 cm) and this was significantly different (P<0.05) from the control which had the highest radicle length. At 8 DAS, the radicle length from the seeds applied with *A. flavus* + *R. nigricans* (72 h) was significantly lower (P<0.05) than from all other treatments. The highest seedling vigour index was observed from uninoculated seeds - control but this was not significantly different (P>0.05) from the inoculated seeds applied with Seedplus®. Seedling vigour index from seeds preinoculated with *A. flavus* + *R. nigricans* for 72 h was the least.

Pot experiment

The result of the effects of types of fungi and duration of preinoculation of *A. flavus* and *R. nigricans* on percentage seedling emergence, shoot and root length and seedling vigour index of physic nut seeds were assessed in the pot are as shown in Tables 4 to 6. Preinoculated seeds treated with Seedplus® recorded the highest seedling emergence (60%) at 5 DAS (Table 4). At 8 DAS, highest field emergence was observed from the seeds applied with Seedplus® and this was significantly higher (P<0.05) than from all other treatments. At 11 and 14 DAS, seeds applied with *R. nigricans* and *A. flavus* + *R. nigricans* for 72 h respectively were significantly lower than those applied with Seedplus®. From 5 to 14 DAS, seedling emergence of seeds applied with *A. flavus* + *R. nigricans* 72 h were less than 50%.

At 5 DAS, shoot length from uninoculated seeds (control) was significantly higher than (P< 0.05) from all other treatments. At 11 DAS, shoot length of seeds applied with *R. nigricans* (72 h) was the lowest. At 14 and 17 DAS, shoot length from uninoculated seeds (control) was the highest. At 20 DAS, highest shoot length (14.30 cm) was observed from the inoculated seeds applied with Seedplus® while the least shoot length was from the seeds preinoculated with *R. nigricans* for 72 h. At 20 DAS, the lowest root length (0.37 cm) was observed from seeds preinoculated with *R. nigricans* (72 h) and this was significantly different (P< 0.05) from the seeds applied

Table 4. Effects of *A. flavus* and *R. nigricans* and the duration of preinoculation on physic nut seeds on seedling emergence.

Treatment	% Seedling emergence at day after sowing			
	5	8	11	14
A. flavus (72 h)	20.00^{b}	26.70^{b}	33.30^{b}	60.00^{ab}
A. flavus (0 h)	20.00^{b}	53.30^{b}	60.00^{ab}	66.70^{ab}
R. nigricans (72 h)	33.30^{ab}	46.70^{b}	53.30^{ab}	46.70^{b}
R. nigricans (0 h)	33.30^{ab}	53.30^{b}	73.30^{ab}	73. 30^{ab}
A. flavus+ *R. nigricans* (72 h)	20.00^{b}	23.30^{b}	30.00^{b}	36.70^{b}
A .flavus +*R. nigricans* (0 h)	23.30^{b}	40.00^{b}	53.30^{ab}	60.00^{ab}
A.flavus +*R. nigricans* +Seed plus®	60.00^{a}	7 3.30^{a}	80.00^{a}	90.00^{a}
Control	50.00^{ab}	56.70^{b}	66.70^{ab}	73.30^{ab}

Column means followed by common letter(s) are not significantly different (P > 0.05) by DMRT.

Table 5. Effects of *A. flavus* and *R. nigricans* and the duration of preinoculation on physic nut seeds on shoot length.

Treatment	Shoot length (cm) day after sowing					
	5	8	11	14	17	20
A. flavus (72h)	1.10^{b}	2.30^{ab}	5.78^{abc}	9.98^{abc}	10.00^{ab}	10.20^{ab}
A. flavus (0 h)	0.90^{b}	1.20 ^{b}	3.20^{bc}	4.70^{bc}	4.80^{c}	4.90^{bcd}
R.nigricans (72h)	0.85^{b}	0.92^{b}	2.10^{c}	3.40^{c}	4.10^{c}	4.58^{cd}
R. nigricans (0h)	0.90^{b}	2.00^{ab}	8.30^{ab}	10.70^{abc}	10.80^{ab}	10.90^{ab}
A.flavus + *R. nigricans* (72 h)	1.00^{b}	1.90^{ab}	3.10^{bc}	5.20b^{c}	7.20^{ab}	10.30^{ab}
A. flavus+ *R. nigricans* (0 h)	0.90^{b}	1.30^{ab}	3.95^{abc}	6.20^{abc}	7.20^{bc}	8.20^{bc}
A. flavus +*R. nigricans*+ Seedplus®	1.80^{a}	3.30^{a}	8.80^{a}	12.70^{ab}	12.10^{a}	13.30^{a}
Control	1.20^{b}	2.00^{ab}	7.70^{a}	12.70^{a}	14.70^{a}	14.80^{a}

Column means followed by common letter(s) are not significantly different (P > 0.05) by DMRT.

Table 6. Effects of *A. flavus* and *R. nigricans* and the duration of preinoculation on physic nut seeds on root length and seedling vigour.

Treatment	Root length (20 DAS)	Vigour index (× 100)
A. flavus (72 h)	3.88^{a}	0.79^{bc}
A. flavus (0 h)	2.22^{a}	0.52^{c}
R. nigricans (72 h)	0.37^{b}	0.20^{d}
R. nigricans (0 h)	4.04^{a}	1.05^{bc}
A. flavus + *R. nigricans* (72 h)	2.35^{a}	0.38^{d}
A. flavus+ *R. nigricans* (0 h)	4.08^{a}	0.43^{cd}
A. flavus +*R. nigricans* +Seedplus®	4.23^{a}	1.15^{bc}
Control	4.72^{a}	1.89^{a}

DAS, Days after sowing. Column means followed by common letter(s) are not significantly different (P> 0.05) by DMRT.

with *A. flavus* (0 h). The root length of uninoculated seeds (control) was the highest but was only significantly higher (P< 0.05) than the seeds applied with *A. flavus* + *R. nigricans* (72 h). Seedling vigour index from the seeds in the control was significantly higher than from all other treatments (P<0.05).

DISCUSSION

The use of *A. flavus* and *R. nigricans* for inoculation was earlier reported by Dayan (1986) that common pathogenic fungi attacking stored seeds belong to the genera Aspergillus, Penicillum, Rhizopus, Mucor and

Fusarium. The adverse effect of *A. flavus* and *R. nigricans* on physic nut seeds might be due to softening and necrosis of the tissue resulting in poor seed viability, low germination and retardation of seedling growth (Mehrotra and Aggarwal, 2003).

Ingold (1991) opined that most seed-borne fungi cause no infection of the seed itself but infect seedlings in the nursery or field. The fact that the seeds applied with *A. flavus* + *R. nigricans* for 72 h had the least percentage germination and vigour index indicated the severity of combined pathogenic effects of the two fungi on physic nut seeds as was observed by Bonner et al. (1994). It was indicated that the longer the inoculum load on the seeds, the higher the pathogenic effect on the seedlings. Sharma and Alan (1998) reported that rapid multiplication of fungal propagule takes place with time in the presence of certain nutrients and water.

Inoculated seeds treated with Seedplus® had relatively higher percentage germination and seedling emergence next to the control. This agreed with the report of Mohannan and Sharma (1991) that systemic fungicides are effective against deep seated seed-borne fungi pathogen. Seedplus® fungicide with systemic action could inhibit the fungal spore development.

Conclusion

It was indicated that *R. nigricans* adversely affected germination and seedling vigour of physic nut seeds than *A. flavus*. Also there appeared to be pathogenic synergism as a result of the presence of both *A. flavus* + *R. nigricans* resulting in lower seedling vigour. However, seed dressing fungicide - imidacloprid, metalaxyl and carbendazim (Seedplus®) was found to be highly effective in the control of the two test-fungi on physic nut seeds as there was no significant difference ($P>0.05$) between the emergence, vigour index of the Seedplus® treated seeds and that of the control. In order to obtain vigorous seedlings, seed-dressing fungicide such as Seedplus® or certified disease-free physic nut seeds should be used for sowing. Further investigation on the effect of seed-borne fungi of physic nut under field condition should be carried out.

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