Nigerian Journal of Ecology (2015) 14: 81-89.

ISSN: 1116-753X

BENOMYL AND FERNASAN-D EFFECTS ON THE INCIDENCE OF SEED-BORNE FUNGI OF COWPEA [*Vigna uncuigula*ta (L) Walp]

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(Accepted 08 December 2015)

ABSTRACT
This study was conducted to evaluate the effect of Benomyl, Fernasan-D and the mixture of the two fungicides applied as seed dressing on seed-borne fungal infection, seed germination and seedling emergence of three varieties of cowpea. Species of *Aspergillus, Curvularia, Corynespora, Fusarium, Alternatia, Rhizopus* and *Penicillium* were isolated from the untreated seeds in Blotter tests. The seed dressing used singly or as a mixture reduced percentage seed infection even at the low rate of 1.5g/kg seed treatments. Reduction in infection was accompanied by a corresponding increase in seed germination in the blotter and seedling emergence in the potted plants. Infections by the seed-borne fungi were completely eliminated by a mixture of Fernasan-D and Benomyl at 3 g/kg of seed in all the tested cowpea seeds except infections by species of *Rhizopus* (3%) and *Penicillium* (2%) in varieties ITA 2246-4 and K-59. On the other hand, Benomyl significantly controlled *Aspergillus* and *Penicillum* species at all tested concentrations (1.5 to 3.0 g/kg). The combination of benomyl and Fernasan-D proved more potent and effective in controlling the seed-borne fungal pathogens of cowpea. This is therefore recommended for use as seed dressing.

Keywords: Seed borne, Seed dressing, benomyl, Fernasan-D, fungicides

INTRODUCTION
Cowpea (*Vigna unguiculata* (L) Walp), a member of Leguminosae (Fabaceae) and sub-family Papilionoidae had its origin from Africa and Asia while it has been known in India since Sanskritic times (Ilodibia *et al*., 2013).

Cowpea serves as good source of plant protein for both tropical and subtropical regions of the world. The crop is used for human food and for livestock feed and an important popular pulse crop throughout India and Africa. Fresh seeds and immature pods may be used as vegetables, frozen or canned as sometimes done in the United State of America. Young shoots and leaves are eaten as spinach; at times it may be dried and stored for use during off-season periods. Cowpea is useful as fodder plant for hay, silage, green manure and cover crops for controlling wind and water erosion (Derek, 1976; Purseglove, 1988).

Despite the importance of this crop, the yield in Nigeria is low, rarely more than 200 kg/ha compared to other African countries (Soyinka *et al.*, 2005). This may be due to poor seeds, cultivation system, seed borne diseases, poor knowledge of modern agronomic technologies and climatic condition. Global yield losses due to plant diseases have been estimated to be 12% at the potential production, which is equivalent to a monetary loss of 50 billion dollars at the producer's level or 550 million metric tons in terms of quantity (Erwin, 1979). This represents a staggering investment in terms of money with regard to production loss in terms of time and materials required to combat the causal organisms. The organisms reduce crop yield, lower quality, and increase cost of production.

Cowpea is the most accepted and most widely cultivated legume in Nigeria, being found in every ecological zone of the country. It is raised from seed and grown primarily as a rainfed crop, though in some parts of North Eastern Nigeria, it is grown on receding flood plains in the dry season. Nigeria accounts for about 70% of the world's total production of this crop (Rachie and Rober, 1974; Quin, 1997; Singh *et al.,* 2003).

This crop is attacked by quite a varied number of fungal pathogens, some of which are seed borne. Many of those seed borne organisms have the ability to deteriorate the seed to the extent that no germination takes place and at times, when germination occurs, the growth may not be vigorous enough to allow the emergence of the seedling from the soil. Seed borne fungi associated with cowpea seeds have been extensively studied in the former western part of Nigeria representing Oyo, Osun, Ondo, Ekiti and Ogun States (Esuruoso, 1975) and species of *Aspergillus, Alternaria, Botryodiplodia, Chactomium, Cladosporium, Collectotrichum, Corynespora, Curvularia, Fusarium, Penicillium* and *Pythium* were isolated and identified. These fungi are known to cause different cowpea diseases ranging from seedling mortality to pod rot in the forest region of Southern Nigeria (Williams, 1975; Singh and Allen, 1979; Bosah, 2013).

Benomyl (Benlate) and Fernasan-D are among the fungicides evaluated for seed-borne fungi of rice *in-vitro* and *in-vivo* at Port Harcourt, Nigeria. Results obtained showed significant inhibition of the seed borne fungi with significant increase in seed germination and seedling emergence of over 70% at all evaluated concentrations but benomyl was reported to be superior to others with higher yields in the field (Ibiam *et al.,* 2005).

Inspite of the huge losses caused by these seed-borne organisms, reinforced by their soil-borne counterparts, little or no seed dressing treatment is being practiced especially by the local farmers who constitute large proportions of the practicing farmers in Nigeria. This study was carried out specifically to determine suitable chemicals for dressing various seeds, especially cowpea, before sowing to reduce the losses due to seed borne organisms and at the same time protect the growing seedlings from some soil-borne fungal pathogens which are known to cause silent and untold losses to farmers.

MATERIALS AND METHODS

This study was conducted in a green-house at the University of Ibadan, Ibadan, Nigeria. Three cowpea varieties, namely Ife-brown, ITA 2246-4 and K-59, obtained from the National seed services Iwo Road, Ibadan were used. Different quantities of the seed-dressing chemicals (Fernasan – D, Benomyl and the mixtures of the two) were weighed and applied to the seeds at the rate of 1.5g, 2.0g, 2.5g and 3.0g/kg of the seeds. Treated seeds were planted on a blotter with three layers of distilled water moistened filter paper in petri-dishes. Each treatment consisted of 200 seeds replicated 20 times (10 seeds per petri dish), which were randomly placed on tables using a randomized complete design and incubated in the laboratory at 21 ± 1°C under 12 hours light and 12 hours darkness. Seeds not treated with seed dressing served as control.

Effect of the seed dressing chemicals on the seed borne fungal infection was assessed by the Blotter method as described by International Seed Testing Association (ISTA, 1966). Observations for any infection were made 7 to 8 days after incubation. For seed germination count, the above procedure was followed but incubation was at 25 to 27°C in the laboratory and germination counts were taken 7 to 8 days after sowing.

Seedling emergence was studied using 26 x 24 x 17 cm plastic pots filled with (2 to 3 cm) top oven sterilized garden soil placed in a green-house of the Teaching and Research Farm at the University of Ibadan in a completely randomized block design and each was replicated four times. Thereafter, germination counts were taken 10 days after sowing.

STATISTICAL ANALYSIS

Data on effects of the fungicides on percentage fungal infection on the three cowpea varieties were presented in graphs while data on percentage seed germination and seedling emergence were subjected to analysis of variance (ANOVA) and significant means were separated by Duncan New Multiple Range Test at 5% probability level.

RESULTS

Effect of seed-dressing fungicides on percentage fungal infection in the cowpea

Varieties: The effects of fungicidal seed treatment on percentage fungal infection in three cowpea varieties: Ife brown, ITA 2243-4 and K-59, under laboratory conditions are presented in Figures 1-3 where species of *Aspergillus*, *Fusarium*, *Curvularia*, *Alternaria*, *Corynespora*, *Rhizopus* and *Penicillium* showed decreased percentage infections by different species of the fungi isolated from the seeds with increase in the concentrations of the fungicide applied. At 3g/kg seed, infections were completely eliminated by a mixture of Fernasan-D and Benomyl (Fig 3a – b) in the tested cowpea varieties except for infection by species of *Rhizopus* (3%) and *Penicillium* (2%) on ITA 2246-4 and K-59 respectively. Percentage infection varied with the isolates in the treated seeds while control seeds had higher percentage infections which varied with varieties and species of fungal isolates.

Table 1: Effect of Fernasan-D and Benomyl on percentage seed germination % germination at indicated seed-dressing rate (g/kg)

Cowpea variety	Control	Fernasan-D (FD)				Benomyl (B)				FD LB			
Concentration	**0.0**	**1.5**	**2.0**	**2.5**	**3.0**	**1.5**	**2.0**	**2.5**	**3.0**	**1.5**	**2.0**	**2.5**	**3.0**
Ife Brown	42g	50ef	56cd	62bc	65b	49f	54de	58cd	60c	62ab	66bc	68ab	70a
ITA 2246-4	45e	48ef	52df	55cd	58bc	48ef	53d	53b	61b	55c	62b	67a	70a
K-59	35h	40g	46f	53cde	65bcd	40g	46f	57de	65be	41g	50c	55ab	62a

% germination was based on a total of 200 seeds/treatment

Values in rows followed by the same letter are not significantly different from each other at P 0.05 (Duncan's new multiple range test)

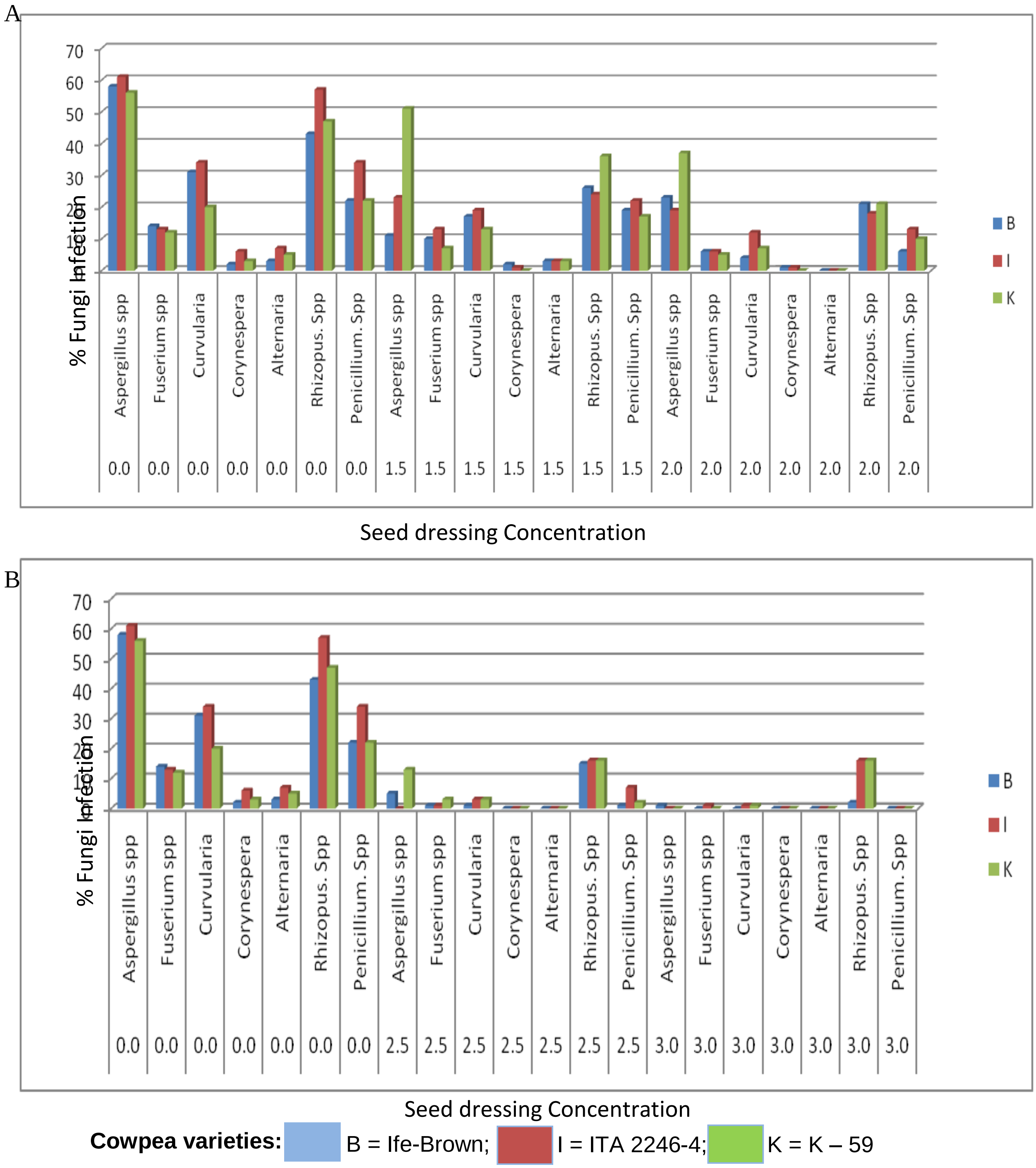

Fig.1a & b: **Effect of Fernasan-D on percentage fungal infection in the three cowpea varieties % infection was based on 200 seeds/treatment**

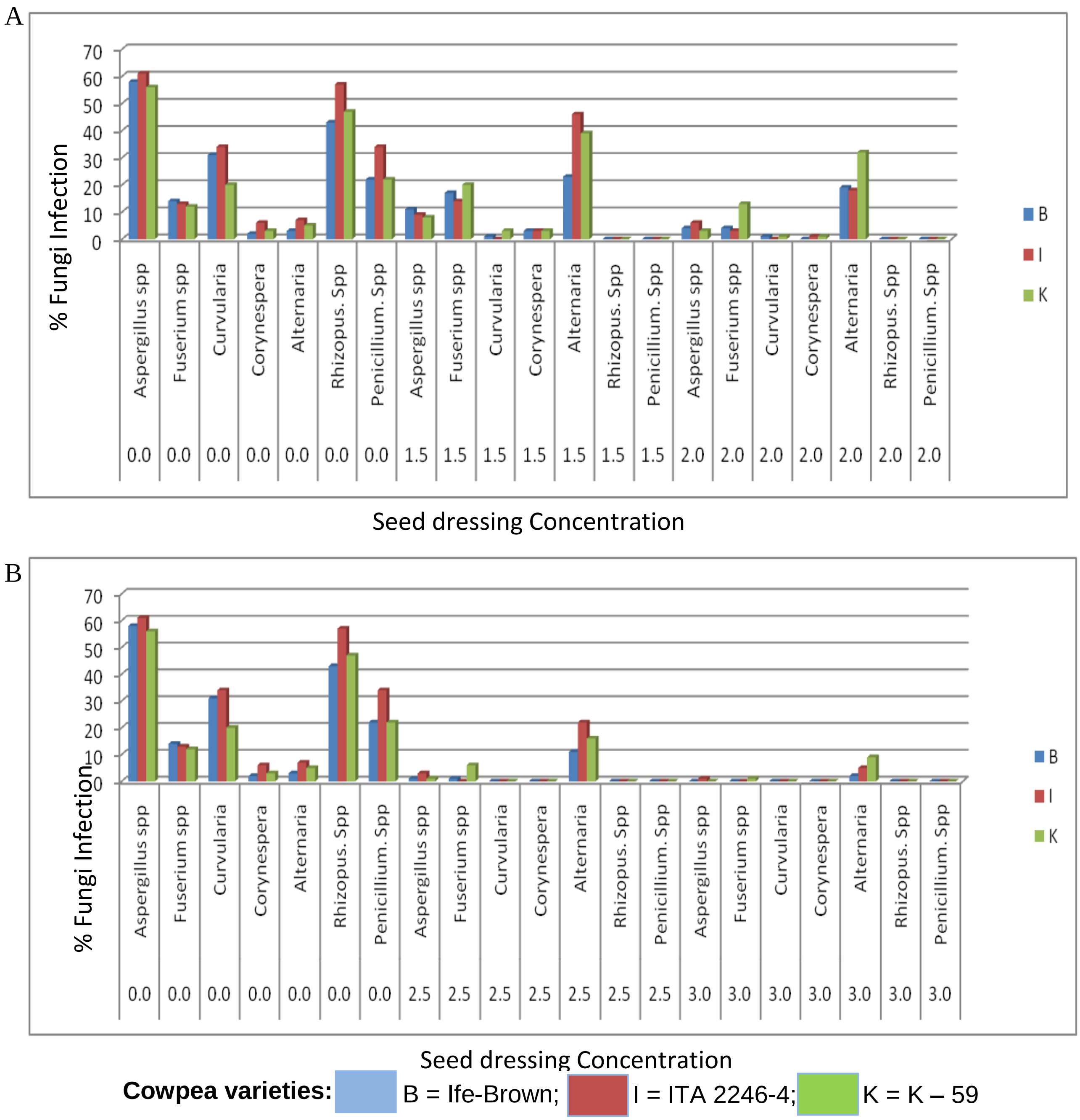

Fig 2a&b: **Effect of Benomyl-D on percentage fungal infection in the three cowpea varieties % infection was based on 200 seeds/treatment**

A

B

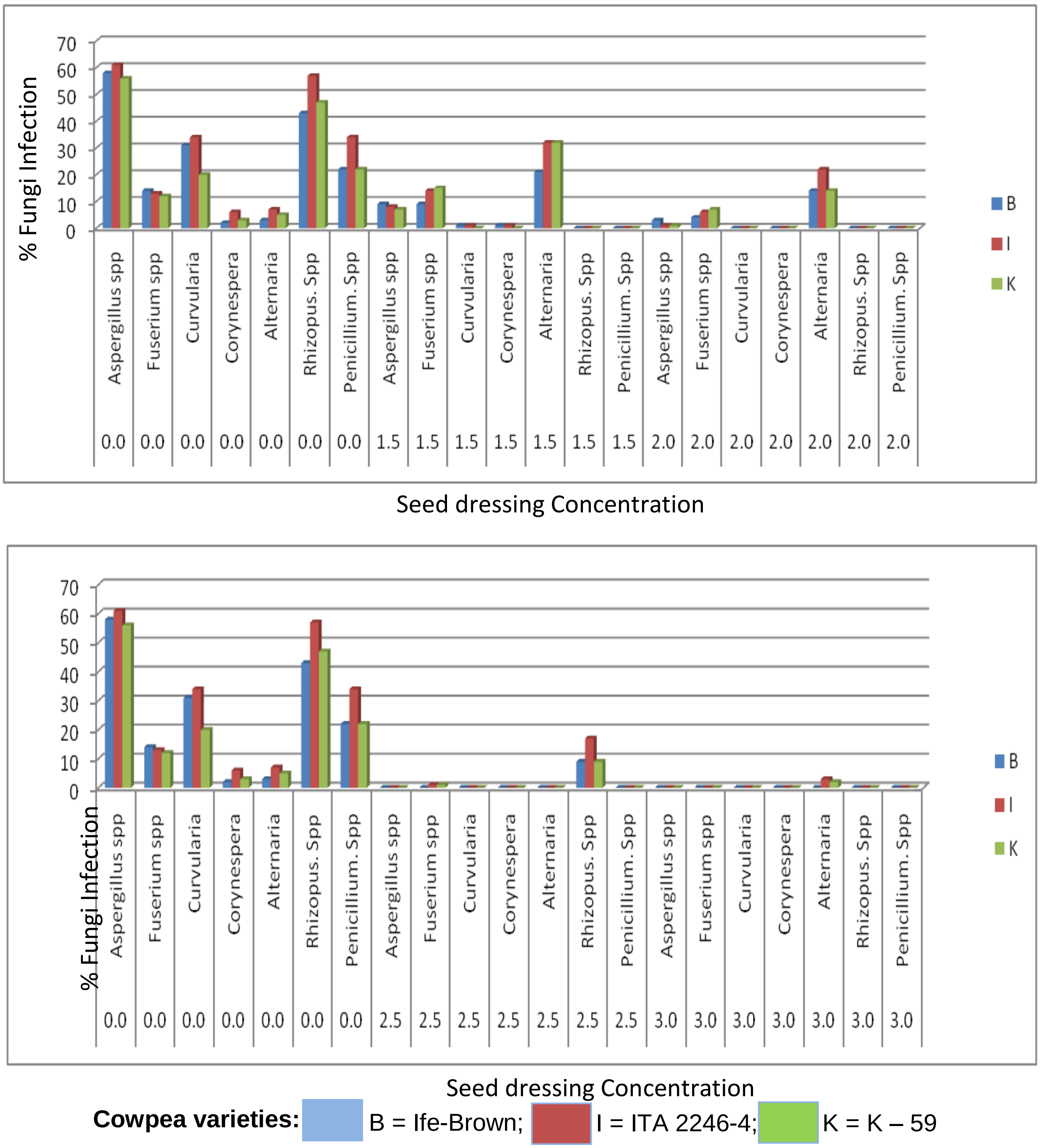

Fig. 3a&b: Effect of Fernasan-D +Benomyl-D on percentage fungal infection in the three cowpea varieties % infection was based on 200 seeds/treatment

Effect of Benomyl and Fernasan – D on Percentage Seed Germination:

Benomyl and Fernasan-D had a significantly positive effect on seed germination (Table 1) while germination in untreated control seeds was significantly lower than in treated seeds at all tested concentrations. At 1.5 and 2.0g/kg seed, Ife brown gave comparable germinations which was significantly lower (P > 0.05) than germination percentage obtained for a mixture of Benomyl and Fernasan-D. The admixture of Benomyl and Fernasan-D gave the highest germination at 3.0g/kg seed which was not significantly higher (P > 0.05) than percentage germinations obtained at either 2.0 or 2.5g/kg seed.

Germination percentages obtained in ITA 2246-4 at 1.5 and 2.0g/kg were not significantly different but were significantly lower (P < 0.05) than respective percentage germinations obtained with the mixture of Benomyl and Fernasan-D. A mixture of the two seed dressings at 2.0 g/kg also gave comparable germination which was not significantly different from either of the two used singly at 3.0 g/kg. However, at 3.0 g/kg seed, a mixture of Benomyl and Fernasan-D had highest germination percentage which was not significantly higher (P > 0.05) than what was obtained at 2.5 g/kg seed.

K-59 followed almost the same trend like the two varieties already discussed. There were no significant differences at concentrations of 1.5 to 3.0 g/kg seed when both Benomyl and Fernasan-D were used singly but when the admixture of the two was applied at 3.0g/kg seed, germination increased significantly by 24%, 7%, and 21% for ife brown, IYA2246-4 and K-59 respectively.

Effect of Benomyl and Fernasan-D on percentage seedling emergence: The effect of Fernasan-D and Benomyl on percentage seedling emergence is shown in table 2. Emergence of seedlings in all the untreated varieties was significantly lower (P < 0.05) than emergence in all the treated seeds probably as a result of other soil-borne pathogenic organisms. Treatment with fernasan-D at the rate of 2.5g/kg was as effective as the manufacturer's recommended rate of 3.0g/kg. In all, a mixture of Benomyl and Fernansan-D at 3.0g/kg seed resulted in a significantly higher seedling emergence in ITA 2246-4 and K – 59 than either of Benomly or Fernasan-D applied singly at the same rate.

Table 2: Effect of Fernasan-D and Benomyl on percentage seedling emergence % germination at indicated seed-dressing rate (g/kg)

Cowpea variety	Cont-rol	Fernasan-D (FD)				Benomyl (B)				FD + B			
Concentration	**0.0**	**1.5**	**2.0**	**2.5**	**3.0**	**1.5**	**2.0**	**2.5**	**3.0**	**1.5**	**2.0**	**2.5**	**3.0**
Ife Brown	38g	48ef	53cd	56bc	58ab	44f	49dc	53cd	58ab	57bc	59ab	60ab	62a
ITA 2246-4	39f	41f	48c	53cd	55bd	48a	52dc	54dc	57bc	48c	41dc	58b	66a
K-59	28f	37c	47c	51bc	54b	37c	40dc	42d	47c	36c	44dc	52b	59a

% germination was based on a total of 200 seeds/treatment

Numbers in rows followed by the same letter are not significantly different from each other at P 0.05 (Duncan's new multiple range test)

DISCUSSION

The results revealed the importance of fungicides in general as a means of boosting and improving agricultural production. Both fungicides, Benomyl and Fernasan-D, expressed their effects on various fungal isolates but Benomyl according to this study, proved to be a better seed-dressing fungicide against species of *Aspergillus* and *Penicillium* at all tested concentrations as against heavy infections in all the control seeds. Reduction in percentage infection was concentration-dependent. Percentage infection decreased as the concentration of the fungicides increased but the potency and spectrum of activity was enhanced when the two chemicals were mixed. The mycelia and spore growth inhibition of the seed borne fungi as a result of the seed-dressing fungicides significantly improved the percentage germination of the treated seeds. Application of these fungicides significantly increased percentage seedling emergence in potted soil. Seed germination observed under laboratory conditions was generally higher than under potted-soil conditions for all treatments, which was in turn higher than the control. The difference in Blotter and potted soil conditions suggests that the various fungicides might be playing a role in suppressing the soil-borne pathogens that have depressing effects on seedling emergence (Fernando, 1980; Imolehin, 1983; 1998). The results obtained agreed with Ibiam *et al.,* (2005) findings where significant increase of over 70% in seed germination and seedling emergence of rice variety (Faro 12) both *in-vitro* and *in-vivo* trials with Benlate and Fernansan-D treated rice seeds were recorded in Port Harcourt. The disparity in germination percentages observed in the three varieties could be attributed to differences in the mycoflora of the seeds. Similar observation was made by Esuruoso (1971) who reported significant increase in seedling emergence of maize, cowpea and groundnut under field conditions when seed – dressing fungicides were applied.

With more research on suitable and effective seed-dressing fungicides, it is hoped that farmers will be relieved of the hazards and drudgery of frequent and regular fungicidal applications which may be injurious to the user's health, of the environment and perhaps reduce the negative influence pathogens resistance in the field soil to the chemicals.

ACKNOWLEDGEMENTS

I wish to acknowledge the assistance of Mrs. Atolagbe of National Seed Services, Iwo Road, Ibadan, who made the cowpea seeds used for the study available.

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