# Impacts of Duration of exposure to Phytochemicals on Seedling Emergence and Vigour of Cowpea (*Vigna unguiculata* L. Walp) Seeds

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***ABSTRACT***

*Seed-borne mycoflora pose serious threats to agricultural production. They can severely affect seed viability and germination, seedling vigour, crop performance, quality and yield. Seed treatments are thought to be a cheap and veritable means for their control. However, owing to concerns about ecological and mammalian toxicities, use of synthetic pesticides is being down-played in crop production. As a result alternatives and/or at least complements to synthetic fungicides are being sought in recent times. This study assessed in vivo the effects of 7 different time regimes (0, 5, 10, 15, 20, 30, 60 Minutes) of exposure to Piper guineense L. as seed-dressing phytochemicals on the seedling emergence and vigour of cowpea (Variety Ife Brown) in the field. The seeds were soaked separately in same concentration of the aqueous extract at different time regimes, removed and air-dried at room temperature for 1 h. The treated seeds were then sown in unsterilized soil contained in 10 cm diameter plastic pots. Ten seeds were sown per pot per replicate. The pots were arranged in a randomized complete block design (RCBD). The entire experiment was repeated twice. The results from the study indicated that the indices of seedling emergence and vigour were best at 10 minutes of exposure to the phytochemical; this was closely followed by 15 minutes of contact with the Piper-gleaned compounds while 60 minutes of contact of the seeds with the phytochemical gave the least effect. To maximize cowpea production therefore, growers of cowpea especially in sub-Saharan Africa could dress cowpea seeds in effective concentrations of Piper guineense suspension for 10-30 minutes (with best result at 10 minutes duration of exposure) to improve its seedling emergence and vigour which will ultimately translate to improved harvest and returns on investment.*

***Key Words**: Seed-borne fungi, Phytochemicals, Germination, Piper guineense L., Concentration*

## INTRODUCTION

Agricultural seeds are associated with a wide array of toxigenic and pathogenic mycobiota. Seed health test reports from different parts of Afro-Asia indicated the association of fungi of the genera *Colletotrichum, Fusaria, Aspergilli, Penicillium, Rhizopus, Rhizoctonia,* and *Mucor* with the fruits of chilli pepper, cereals such as sorghum and maize as well as legumes including French bean and cowpeas (Makun et al., 2012; Syed et al., 2012; Suleiman and Omafe, 2013). Various workers in India and Nigeria reported the association and contamination of *Botytis cinerea, Penicilliun digitatum, Rhizoctonia solani, Aspegillus flavus, A. niger, Fusarium verticilloides, Curvularia sp., Colletotrichum destructivum and C. truncatum* with cowpea seeds (Makun et al., 2012 Mogle, 2013; Awurum and Enyiukwu, 2013).

The agricultural importance of contamination of agro-seeds with seed-borne fungi has been variously documented. Seed-borne mycobiota affect seed health resulting in poor seed germination, reduced seedling emergence and vigour, poor crop performance, reduced yield and in some cases total crop failure. These effects in the overall impact grain quality, marketability and gross farm economy (Enyiukwu et al., 2015). In a recent field study in Nigeria by Anjorin and Mohammed, (2014), combined presence and high inoculum load of *Rhizopus nigrican* and *Mucor racemosus* on the seeds of water melon (*C. lanatus*) was reported to result in poor germination profile and low seedling vigour of the crop.

Control of pathogenic seed-borne mycobiota of agricultural crops has been successfully attempted with synthetic seed chemicals or field sprays. However, seed treatment is preferred over field sprays because relatively low amounts of active ingredients are required for seed treatment compared to field sprays; and on the other hand applications can be done in contained areas unlike in field sprays (Van der Wolf et al., 2007; Enyiukwu et al., 2015). This therefore, reduces the associated health risks and drift-borne hazards from the crop protection sprays. For instance, Awurum (2011) reported from culture studies that seeds of wing bean surface-sterilized with 10% sodium hypochlorite germinated better than untreated controls. While SeedPlus® a synthetic fungicide improved the germination and seedling vigour of water melon in a field trial in Niger state Nigeria (Anjorin and Mohammed, 2014); Apron Star® strongly impeded growth and mycelia biomass accumulation of both *Aspergillus niger* and *Fusarium verticilloides* on dressed cowpea seeds by 93% (Makun et al., 2012).

These successes notwithstanding, besides disrupting the food chain, residues from synthetic seed-treatment fungicides such as Captan and Thiram used for dressing large volumes of various seeds have been implicated in some forms of allergies, cancers, birth defects etc. which ultimately led to their ban (Enyiukwu and Awurum, 2011; 2012). Several investigators have reported phyto-pesticides as veritable user-friendly complements and/or viable alternatives to synthetic pesticides (Mogle, 2013; Bassey et al., 2013). Results from a trial indicated that the phyto-pesticide Biosept (33% grapefruit extract) impeded the development of fungi on some vegetables as effectively as a synthetic fungicide Sarfun T65DS (Szopinska et al., 2007). And similarly, soaking cowpea seeds in suspensions of Agemone mexicana, Semacarpus anacardium prior to sowing, were superior to benomyl in inhibing the seed-borne fungi of cowpea (Mogle and Maske , 2012). In all, these measures improved seed germination. For instance, a trial on maize seeds with ethanolic neem leaf extracts, accoding to Anjorin et al. (2008) significantly improved its germination to 100% and seedlings vigour index by 5.4 compared to 30% and 0.93 respectively obtained from the controls inoculated with metabolites from the toxigenic and pathogenic *Fusarium verticilloides.*

Nevertheless several factors have been reported to affect the efficacy of plant-derived pesticides. These include time of application, kinds of microorganisms in the rhizosphere, UV-radiation, and degrees of temperature and humidity. Others are type of plant materials, concentration of application and duration of exposure to the toxicant. We have previously reviewed the influence of extracting solvents and particle size on concentration of plant derived chemicals (Enyiukwu et al., 2014) And have also reported the influence of formulation, type of plant materials as well as concentration on both the seed-borne mycobiota and germination of treated cowpea seeds (Awurum and Enyiukwu, 2013; Awurum and Ucheagwu, 2013; Enyiukwu *et al*., 2015). This present paper assessed the impact of different regimes of exposure to Piper-derived fungicides on the germination and seedling emergence of cowpea (*Vigna unguiculata* L. Walp.) seeds.

## MATERIALS AND METHODS

### Sources of seeds and plant materials

The experiment was conducted in a micro-plot at the Research and Training (R & T) Farm of the Crop and Soil Sciences of the Michael Okpara University of Agriculture, Umudike. The seeds of cowpea variety Ife Brown sourced from the same Unit (R&T) of the University; and dried seeds of *Piper guineense* L. obtained from Umuahia main market, were used in the study.

### Preparation of plant extracts

Dried seeds of *Piper* guineense L. were air-dried on the laboratory bench at room temperature of $27^{o}$ C overnight. About 15g of the seeds were weighed out and ground into powder, using an electric blender (Model: XL 15). Thereafter 10 g of the powdered specimen (*Piper guineense* L. seeds) was weighed out using a sensitive electric balance. The powder was put into a 200 mL beaker to which 100 mL of sterile distilled water was added and allowed to stand for 1 h. At the end of the hour, the paste was strained through 4-folds of sterile cheese cloth, to obtain the filtrate corresponding to 10% strength of the plant material.

### *In vivo experiment*

One hundred and eighty (180) cowpea seeds (Ife Brown) were soaked in the aqueous suspension of the 10% strength of the filtrate of the plant material for varying periods of time (5, 10, 15, 20, 30, 45, and 60 minutes), removed and air-dried at room temperature ($27^{o}$C) for 1 h. At the end of this period, 30 seeds soaked for a particular length of time in the aqueous phytochemical were then sown in the Plastic pots and incubated for 5 days at $27^{o}$C, and then observed for germination and seedling emergence. Ten (10) cowpea seeds were sown per pot per replicate. The control was set up in a similar manner but consisted of cowpea seeds soaked in sterile distilled water for the corresponding lengths of time. The whole experiment was repeated twice giving 60 seeds per treatment and 360 seeds for each set of observational units. The plastic pots were arranged in a randomized complete block design (RCBD) in the micro plot. At the end of 7 days, counts of seedlings that have successfully emerged and established from the pots were taken per exposure time per replicate; and means of replicates were recorded. Records of the individual seedling vigour were also taken per treatment per replicate.

The percentage seedling emergence from the treated cowpea seeds was assessed based on the modified formula by Anjorin et al. (2008) as:

**% Seedling emergence** = Number of seedlings that emerged successfully from the treated cowpea seeds/Total number of cowpea seeds sown x 100/1

The influence of the duration of exposure of the cowpea seeds to the piper-derived phytochemical was also assessed by visual assessment of the individual seedling height, stem girth, leaf size, number and colour according to the modified form of the formula by Amadioha (2004) as:

- 1 Seedling has very poor vigour
- 2 Seedling slightly vigorous
- 4 Seedling moderately vigorous
- 6 Seedling highly vigorous
- 8 Seedlings very highly vigorous

The influence of the duration of exposure of the cowpea seeds to the *Piper*-derived phytochemical was evaluated as improvement in the vigour of the seedling from seeds treated to varying durations in the suspension of the phytochemical compared to the control (water).

## STATISTICAL ANALYSIS

All measurements collected from this study were 3 replicates and were analyzed by simple percentages and analysis of variance (ANOVA) using Genstat 2009 version, at 5% level of significance. Fisher's least significant difference (FLSD) at $p<0.05$ was applied to assess the differences amongst the means.

## RESULTS

The result presented in Table 1 showed the emergence profile of the treated cowpea seeds. It indicated that the treatments had profound influence on the seedling emergence and vigour. At 10 minutes of exposure time, the most emergence and highest seedling vigour were obtained in the study. This was slightly superior to the effects of the treatments at 15 minutes of exposure. However, the least emergence and seedling vigour was recorded at the exposure of the seeds to the phytochemicals for 60 minutes (Table 1)

**Table 1: Effects of the duration of exposure to *P. guineense L.* on the seedling emergence and vigour of the dressed cowpea seeds**

| Duration of Exposure to the Phyto-chemical | Mean seedling emergence (%) | Mean Seedling vigour |
|---|---|---|
| Soaked for 5 minutes | 73.33 | 3.67 |
| Soaked for 10 minutes | 80.00 | 6.68 |
| Soaked for 15 minutes | 76.00 | 5.73 |
| Soaked for 20 minutes | 80.01 | 5.45 |
| Soaked for 30 minutes | 73.33 | 5.02 |
| Soaked for 60 minutes | 49.67 | 2.67 |
| Soaked for 0 minutes | 46.09 | 1.25 |
| **LSD** | **4.27** | **0.98** |

*Data are means triplicate determinations

## DISCUSSION

Several workers have reported the association of species from many fungal genera with the seeds of agricultural crops. Chigoziri and Ekefan, (2013) reported the association of Colletotrichum capsici and Aspergillus flavus with Chilli pepper in Benue State, Nigeria, while Penicillium digitatum, Aspergilllus niger, Botrytis cinerea, Rhizoctonia solani, Fusarium spp. Curvularia sp., and Colletotrichum destrcivum and C. truncatum were associated with the seeds of cowpea plant in both India and Southeast, Nigeria (Enyiuwu and Awurum, 2011; Mogle, 2013; ). These toxigenic species reduce seed viability, germination, total crop performance, yield, and produce quality (Awurum and Enyiukwu, 2013; Awurum and Ucheagwu, 2013). Controlling these diseases at the seed-borne phase is seen as the cheapest form of their control (Van der Wolf et al., 2007; Syed et al., 2012). Many workers have reported the efficacy of plant-derived pesticides as effective seed treatments and plant health management chemicals in the recent times. According to Suleiman and Omafe (2013), Phytochemicals from Morinda lucida, Cymbopogon citratus and Riccinus cummunis effectively checked the growth and development of maize kernel-borne *A. niger* and *P. digitatum*. However, these phyto-chemicals were selectively more toxic to *P. digitatum* than *Aspergillus niger*. In both pot and field experiments, Mangang and Chhetry (2012) found Coix lacryma jobi and Lantana camara to effectively decrease the incidence of Rhizoctonia solani-induced rot in French bean. Similarly extracts from Moringa oleifera, Annona muricata, and Vernonia amygdalina inhibited Colletorichum destructivum on cowpea, with the effects of M. oleifera comparing well with benomyl (Akinbode and Ikotun, 2008).

The efficacy of these plant derived chemical as seed treatment fungicides has been found to be influenced by several factors besides rhizosphere microorganisms. These include type of active ingredients (a.i), concentration of application, extractant, combination of active ingredients and formulation of the active principles (Bassey et al., 2013; Awurum et al., 2014; Enyiukwu et al., 2014). As an instance, Bassey et al. (2013) found that ethanolic leaf extracts from Jatropha carcus and Chromolaena odorantum out-performed their aqueous counterparts in effectively minimizing the seed-borne mycobiota of Solanium gilo. A parallel trial on maize also indicated that 9 days after sowing (DAS) ethanolic leaf extacts of neem (Azadirachta indica Juss) improved the seed germination and the seedling vigour index by 100% and 5.4 better than the control at 30% and 0.93 respectively (Anjorin etal., 2008).

The effectiveness of duration of exposure to powdered extracts from some spice plants on the reduction of seed-borne mycobiota and improvement of germination of cowpea seeds were asserted from a 3-month study in Southeast Nigeria by Awurum et al. (2013). In like manner, the impacts of different regimes of exposure of cowpea seeds to between 5-15 h in fungitoxic suspensions from Agemone Mexicana and Semecarpus anacardium was reported from a study in India by Mogle and Maske (2012). These workers concluded that besides concentration and formulation of the pesticides, that duration of exposure to the myco-toxicants played a significant role to reduce the cowpea seed-borne fungi and improved the seed germination and vigour of the seedlings. Findings from this present study showed that the duration of exposure to the phytochemicals had significant effects on the assayed agronomic parameters of germination and seedling emergence of the cowpea plants. In these regards we agree with the afore-mentioned submissions. It appears that the pathogenic mycobiota are borne just beneath the seed testa and outer borders of the endosperm. Soaking the seeds for a small to medium time period eliminates both the mycobiota and their toxins and gives the seeds ample opportunity to germinate and do well. However, prolonged soaking may have allowed for room for the seed embryo to be engulfed and weakened by the toxicant. The mechanism of this action may have been by inactivation of enzymes involved in germination, energy production and growth.

We have previously reported that cowpea seeds could be dressed by immersion in 5-10% concentration (w/v) of Piper guineense extracts (Enyiukwu et al., 2015). To maximize cowpea production therefore, our findings in this present work indicated that good seeding emergence and vigour profile could be attained from cowpea by immersion of its seeds in aqueous Piper guineense suspensions for 10-30 minutes; with best result at 10 minutes of exposure.

## CONCLUSION

Cowpeas are important sources of dietary protein, carbohydrate and fibers which play roles in the nutrition of sub-Saharan African people. Its production is however, seriously challenged by high fungal disease pressures especially seed-borne mycobiota. Seed treatment is seen as effective and cheapest form of their control strategy. Several synthetic seed treatment fungicides are been de-emphasized due to mammalian toxicity and fungal resistance. Plant derived compounds are a veritable and viable alternative and complimentary myco-toxicants for disease control. Their efficacy and phyto-toxicity in some cases, however is influenced by three key factors of nature of active ingredient, rate/concentration of application and duration of exposure to the toxicant. Herein we report the impact of different regimes of exposure (5-60 minutes) on the germination and seedling emergence of cowpea. And drew a data-supported conclusion, that immersing cowpea seeds in 10% concentration of aqueous Piper guineense suspension for 10-30 minutes prior to sowing; significantly improved its germination and seedling emergence. Hence, growers of the crop can effectively improve its production by adopting this practice to reduce its fungal load, improve its germination and seedling emergence.

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**Plate 1: Seedling Emergence Profile of the Treated Cowpea Seeds**