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Effect of *Capsicum chinense* fruit suspension on seed germination of some *Capsicum* cultivars (Pepper) grown in Nigeria.

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Abstract

The role of secondary metabolites like capsaicinoids appears to be more of adaptive and protective. Capsaicin, which is found only in the genus *Capsicum,* is responsible for the heat sensation in peppers. Pepper vary in capsaicin content with genotype, and also in seed germination rate. There is therefore the possibility that capsaicinoids could have some effect on seed germination. Germination studies were therefore carried out, in petri-dishes and in soil, on some *Capsicum* varieties in Nigeria to ascertain the effect of external application of freshly grinded suspension of *Capsicum chinense* (yellow Cameroon pepper - one of the hottest peppers) on germination. The generally slow rate of germination in *Capsicum* species (without any treatment application) was confirmed in this study; and *Capsicum annum* var. grossum (Bell pepper) had the lowest germination percentage. The treatments further lowered the germination percentage with increased concentration of the suspension of grinded *Capsicum chinense*. This effect was more remarkable in the germination studies carried out on filter paper, than those done in soil. Capsaicin could therefore be said to affect negatively the germination of *Capsicum* seeds.

Keywords: Capsaicin, capsaicinoid, *Capsicum* varieties, germination rate

Introduction

Chile peppers are one of the first crops domesticated in the Western Hemisphere about 10,000 BCE (Perry et al., 2007). The genus *Capsicum* is native to the Americas. One possible reason for its early domestication is that chile peppers are well known as medicinal plants by indigenous peoples (Cichewicz and Thorpe, 1996). It consists of quite a number of pepper varieties that differ across the continents of the world in phenotype, flavour and the burning sensation they give when eaten (heat). They have been cultivated for thousands of years by many cultures to enhance the flavor and aroma of foods (Bosland and Votava, 2012). In Nigeria, peppers are desired, used and combined in various proportions for making different dishes based mainly on their flavor, heat, aroma, puree content and soup reddening abilities amongst others; and *Capsicum* varieties differ remarkably in these qualities. They are also used for medicinal purposes and as preservatives.

The heat of most *Capsicum* varieties is as a result of the accumulation of a group of related alkaloids known as capsaicinoids that are unique to this genus (Kozukue et al., 2005); and the major one produced in chile peppers is capsaicin- 8-methyl-N-vanillyl-6-nonenamide. In *Capsicum* the heat level is both a qualitative and a quantitative trait. The ability for the fruit to be hot or not can be inherited as a single dominant gene, Pun1 (Pungency) located on chromosome 2. A second gene for lack of heat in the fruit is described in C. chinense and is caused by the absence of vesicles on the placental tissue (Bosland and Votava, 2012). The lack of capsaicin in Bell pepper is due to a recessive form of a gene that eliminates capsaicin and consequently the "hot" taste usually associated with the rest of the *Capsicum* genus (Kumar et al., 2011).The quantitative aspect of heat level is based on the concentration of capsaicinoids that accumulate in the vesicles on the placental walls (Suzuki et al., 1980).

The level of capsaicinoids vary with genotype (Hernandez-Verdugo et al., 2001; Zewdie and Bosland, 2000a), the environment the plant is grown in and with the fruit location on a plant (Zewdie and Bosland, 2000a and b). Germination of seeds of *Capsicum* species are generally slow and also vary with genotype (Demir and Okcu, 2004; Hernandez – Verdugo et al., 2001). Hydro-priming of the seeds of Bell pepper (*Capsicum annum* cv Goliath) for 24 hours has however been demonstrated to enhance germination and seedling emergence (Uche et al., 2016).

A study on gut retention of *Capsicum annuum* seeds in birds, by Tewksbury et al. (2008) showed that increased retention resulted in a greater proportion of seeds germinating, thus suggesting a role for scarification. Capsaicin extends gut retention for most seeds, resulting in greater seed scarification and higher germination rates. Capsaicin is an irritant for mammals (including man) who destroy the seeds with their molar teeth, preventing them from germinating. Chilli pepper consumed by birds on the other hand is advantageous to the plant because it passes through the digestive tract and can germinate later. Thus natural selection may have led to increasing capsaicin production in peppers because it makes the plant less likely to be eaten by animals that do not help disperse it (Tewksbury et al., 2008).

The recent sequencing of the Capsicum genome strongly suggests that capsaicinoid production originated through the evolution of new genes by unequal duplication of existing genes and owing to changes in gene expression in fruit after speciation (Kim et al., 2014). Wide variation in capsaicinoid content has been introduced through domestication into cultivated species of chile peppers.

Since both capsaicinoid levels and germination of *Capsicum* species, vary with genotype, it was thought that there could therefore be the probability of some relationship between the poor germination of *Capsicum* seeds and their capsaicinoid levels. To verify this, germination studies were carried out, both on moist filter paper in petri dishes and in soil, on the effect of the application of freshly grinded suspension of *Capsicum chinense* fruits on some *Capsicum* cultivars grown in Nigeria, but varying in capsaicinoid content.

Materials and Methods

Plant materials
The five *Capsicum* cultivars used for this greenhouse experiment and the symbols used for them were:
Capsicum frutescens –Bird pepper (Atawere) ----SSP
Capsicum annuum var. fasciculatum – Red pepper or Cayenne pepper (Sombo) ---LSP
Capsicum annuum var. grossum – Giant bell pepper (Tatase) ----TP
Capsicum annuum var. abbreviatum – Scent pepper (Atarodo) ------RSP
Capsicum chinense - (Cameroon pepper; Yellow Scent pepper) ---- YSP

The hottest of the five cultivars, which happens to be YSP, was used as the source of capsaicinoid for the experiment. One kilogram of YSP was grinded and made into a one litre suspension which was used as 100%; and 500grams of YSP grinded in one litre of water served as 50% concentration. The large quantity of pepper used is because the capsaicin is just a small fraction of the total body weight of the pepper. Two sets of germination experiments were carried out: the first was on moistened Whatman filter paper in petri dishes, and the second in soil. The experimental design was a randomized complete block, with two treatment levels and a control for each of the five cultivars, done in three replicates each. Ten seeds each were placed on the filter paper, and sown in soil respectively. To those in the petri dish with filter paper, 5ml each of the treatment concentration was added; while to the seeds sown in soil, 100ml each of the treatment concentration was added.

Result

Germination of the *Capsicum* seeds was observed to have commenced from day 4 on moistened filter paper in petri dish and from day 7 in the soil.

Of all the *Capsicum* cultivars studied, TP had the poorest germination rate in those sown on filter paper (Fig. 1 and Table 2). The treatment effect was generally more on the filter paper-sown seeds than in the soil. On filter paper, no germination was seen in TP and RSP for both treatment concentrations; and for YSP, SSP and LSP, some germination were observed with the 50% treatment, but none with 100% except in SSP.

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Table 1. Treatment Effect on the Germination Percentage of *Capsicum* cultivars sown on filter paper

Cultivar	Control	50% Treatment	100% Treatment
TP	43.0	0	0
RSP	56.7	0	0
YSP	86.7	13.3	0
LSP	70.0	53.3	0
SSP	76.7	33.3	0

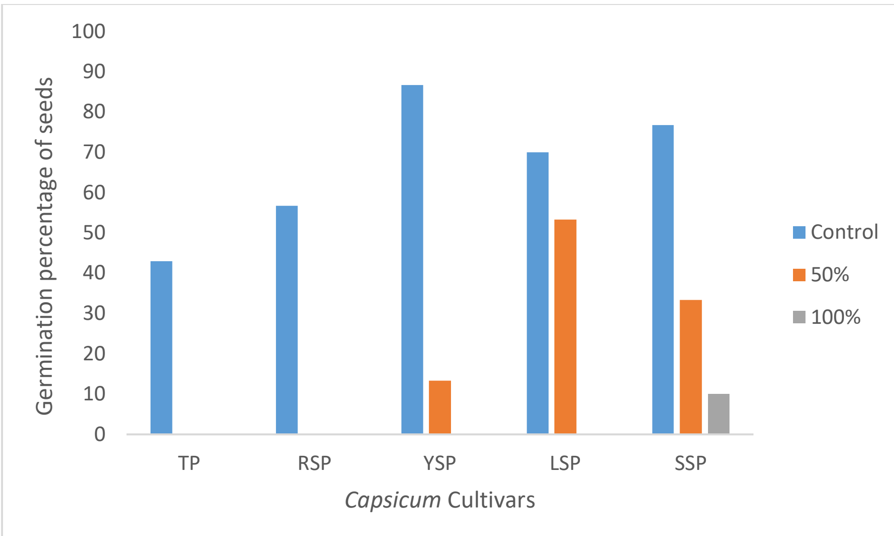

Fig. 1: Comparism of the Treatment effect of the application of *Capsicum chinense* suspension on the Germination of seeds of *Capsicum* cultivars sown on filter paper

In the soil experiment, seed germination was recorded in all treatments for all the *Capsicum* cultivars. Nevertheless, germination in the 100% treatment for all cultivars and some 50%, germination did not commence until day 11 to 14 after sowing, while majority of the others fell on the 8th day. Though there was an increase in germination percentage with RSP, LSP and SSP cultivars at 50% treatment, it dropped at 100%; while those of TP, YSP and SSP dropped from the values got with the Control. In both experiments, YSP has the highest germination percentage with Control.

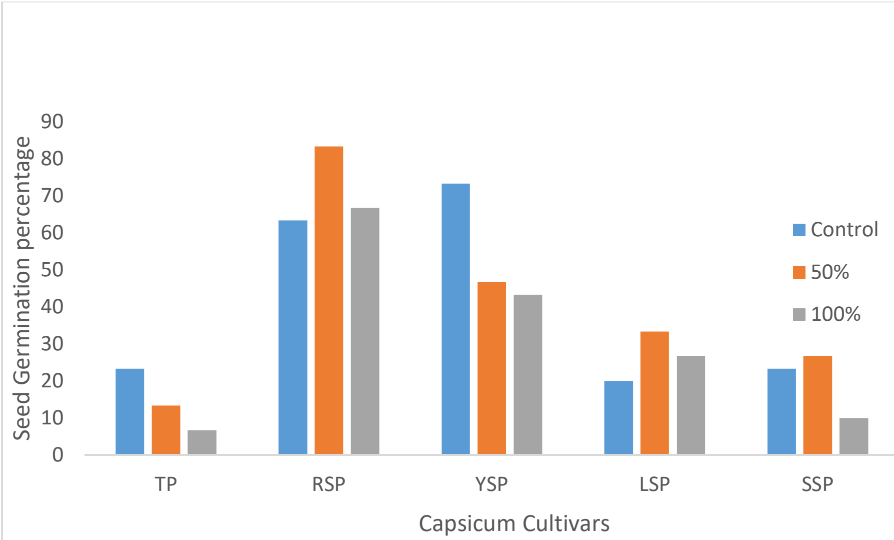

Fig. 2: Comparism of the Treatment effect of the application of *Capsicum chinense suspension* on the Germination of seeds of *Capsicum* cultivars sown in soil

Table 2. Treatment Effect on the Germination Percentage of *Capsicum* cultivars sown in soil

	Control		50% Treatment		100% Treatment	
	EXP I	EXP II	EXP I	EXP II	EXP I	EXP II
Cultivar	F.Paper	Soil	F.Paper	Soil	F.Paper	Soil
TP	43.0	23.3	0	13.3	0	6.7
RSP	56.7	63.3	0	83.3	0	66.7
YSP	86.7	73.3	13.3	46.7	0	43.3
LSP	70.0	20.0	53.3	33.3	0	26.7
SSP	76.7	23.3	33.3	26.7	0	10.0

Discussion

From Fig. 1 it is obvious that the application of the suspension of *Capsicum* chinense negatively affects germination of the seeds of *Capsicum* cultivars sown on filter paper moistened with the treatment concentrations. This pronounced treatment effect is likely due to the direct contact with the seeds, because compared to the soil experiment, the treatment effect is not that pronounced (Fig. 2). Nevertheless, the result of the filter paper sown seeds establishes the fact that capsaicin reduces germination in *Capsicum*. This is in line with the findings of Barchenger and Bosland (2016), who proposed that germination inhibition could be an autopathic effect of capsaicin, and the cause of reduced and slowed germination of some chile pepper seed genotype. The SSP cultivar is the most tolerant of the treatment concentrations in the first experiment, followed by LSP; but in the soil experiment, RSP is the most tolerant, followed by YSP. Though used as the source of capsaicin in this study because of its exceptional heat, the Control for YSP was observed to have the highest germination percentage in both experiments. According to Fujiwake et al. (1982), capsaicinoids are produced during fruit maturation in the fruit placenta, and also in the fruit wall of some *C. chinense* (Jacq.) cultivars (Bosland et al., 2015). This could be the reason why YSP gave the highest germination percentage, despite its exceptionally high heat, as capsaicinoids are not synthesized in or on the seeds (Ancona-Escalante et al., 2013; Fujiwake et al., 1980). The cultivars TP, LSP and SSP all show a drop in germination percentage in soil compared to that on filter paper.

The seeds sown in soil didn't start germinating until the seventh day. The germination rate of *Capsicum* is known to be generally slow due to hard seediness known as seed coat impermeability (Demir and Okcu, 2004).

Seed priming as an effective seed invigouration method has become a common seed treatment to increase the rate of uniformity of germination and seedling emergence and crop establishments in most crops especially in advance countries. According to Khan (1992), seed priming is a controlled hydration process that involves exposing the seed to low water potentials that restrict germination, but permits pre-germination physiological and chemical changes to occur. Hossein (2013) defined seed priming as a controlled hydration process followed by re-drying that allows seed to imbibe water and begin internal biological processes necessary for germination, but which does not allow the seed to actually germinate. Priming of seed promotes germination by repair of the damaged protein, RNA and DNA (Koehler et al, 1997); pre-germination metabolic activities proceed, while radical protrusion is prevented.

The treatment effects on the seeds was more pronounced on the filter paper than in the soil. The direct absorption of the suspension of grinded pepper on filter paper by the *Capsicum s*eeds, gives direct assess of capsaicin to the seed embryo. Barchenger and Bosland (2016) showed that exogenous application of capsaicin to seeds of *Capsicum annuum* did not only reduce germination, but also delayed it. Furthermore, capsaicin is a well-established allelo-chemical, and has been shown to reduce root and shoot growth or suppress germination in several plant species (Kato-Noguchi and Tanaka, 2003; Siddiqui and Zaman, 2005).

Treatment effect of seeds sown in soil wasn't as pronounced as on filter paper, and this could be so because of other soil parameters that must have interacted with the treatment, thus playing down its effect. Nevertheless, the negative effect of capsaicin on seed germination of *Capsicum* is established.

The different *Capsicum* cultivars were also observed to vary in germination rate, with bell pepper (TP) having the poorest. Bell pepper is a cultivar of the *Capsicum annum* (Sweety, 2013); it belongs to the cultivar group known as sweet pepper which is the only *Capsicum* which does not produce capsaicin. The lack of capsaicin in bell pepper is due to a recessive form of a gene that eliminates capsaicin and consequently the "hot" taste usually associated with the rest of the *Capsicum* genus (Kumar et al., 2011). Hermandez-Verdugo et al. (2001) reported also that seed germination rate has been observed to be genotype specific.

Bell pepper in this experiment gave the lowest germination percentages in control and in both filter paper and soil experiments (Fig. 1 and 2). It was observed however that the treatment effect lowered further the germination percentage. Since bell pepper contains virtually no capsaicin (totally tomato) as it is not hot, capsaicin can therefore not be considered as responsible for the poor germination rate observed in the control, rather the hard seediness as the seeds are relatively bigger and firmer than the others. Uche et al. (2016) in their study, using hydro-priming on germination and seedling emergence of Green bell pepper confirms that bell pepper takes a longer time to germinate and emerge (10-14days) compared to other *Capsicum* species (6-8days). They observed further that often, when seedling emergence sets off, it is slow and not uniform; and the delayed emergence also increases the chance of the seedlings being infected by damping-off pathogens. Rapid and uniform germination and emergence of green pepper is important in green pepper production. It is the foundation on which establishment is based and this determines potential yield. A major approach to increase good stand establishment and increase crop production is seed invigoration (Farooq et al., 2006), and seed priming has become an effective seed invigoration method.

The further lowering of the germination percentage by external application of *Capsicum chinense suspension* was observed in all the *Capsicum* cultivars used, especially in the filter paper experiment (Fig 1). In preparing the soil for cultivation therefore, remains of chilli pepper fruits should not be ploughed into the soil, since this could reduce and delay germination of seeds sown; and delayed germination promotes seed rot.

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