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THE EFFECT OF SODIUM HYPOCHLORITE AND ETHANOL AS SEED STERILANTS ON COWPEA INFECTED WITH COWPEA MOTTLE VIRUS

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Abstract

The transmission of viruses through seed can be of considerable ecological significance for virus perpetuation and dissemination. This can also be of economic consequence for the plant as the seeds can be an efficient way by which virus diseases are disseminated in plants. An experiment was conducted to test the efficacy of Sodium hypochlorite and Ethanol at different concentrations as sterilants on cowpea seeds variety 96D-GLO infected with Cowpea Mottle Virus. The cowpea seeds were mechanically inoculated with the virus and surface sterilized at the rate of 5ml inoculum per seed with Sodium hypochlorite (0.5%, 0.75%, 1.0%), Ethanol (75%, 85%, 95%) and distilled water served as control for the experiment. The seeds were then sun dried for 1 hour before sowing into plastic buckets at the rate of 6 seeds per pot. The results indicated that Sodium hypochlorite and Ethanol at different concentrations significantly ($P \geq 0{\cdot}05$) reduced virus disease severity on the plants. This was manifested by increased growth parameters and significantly higher yields compared to the control. The experiment indicated that at the 10th week after planting, Ethanol (95%) had the significantly ($P \geq 0{\cdot}05$) lowest percentage virus disease severity (17.63%), tallest plants (59.13cm), largest number of leaves per plant (72.30) and highest weights for pods (37.17g) and seeds (36.07g). These results indicate prospects for the use of Sodium hypochlorite and Ethanol as seed sterilants in ameliorating viral disease severity on cowpea.

Keywords. Cowpea, disease severity, growth, seed transmission, sterilant, viruses, yield

Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is one of the important grain legumes in the world and plays an important role in the livelihood of millions of people in developing countries (El Naim and Jabereldar, 2010). Cowpea has various uses and it is consumed as grain, leaf and forage with high nutritive value and high palatability (Whitebread and Lawrence, 2006). Cowpea is also an important source of food, income and livestock feed. It forms a major component of tropical farming systems because of its ability to improve the fertility status of marginal lands through nitrogen fixation (Timko and Singh, 2008). It has considerable adaptation to high temperatures and drought compared to other crop species, making it suitable for cultivation in semi-arid areas (Tekle, 2014).

Cowpea is a major source of cheap quality protein for both rural and urban dwellers in Africa (Ajeigbe *et al.*, 2012; Dube and Fanadzo, 2013). The leaves and green pods are consumed as vegetable and the dried grain is used in many different food preparations. Protein content of cowpea leaves range from 27 to 43% and protein concentration of the dry grain range from 21% to 33% (Abudulai *et al.*, 2016).

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Cowpea is however susceptible to a number of fungal, bacterial, and viral diseases. Of more than the twenty viruses reported on cowpea from different areas of the world, eight are known to occur in Africa (Lima *et al.,* 2011) and seed transmission is said to account for about one-seventh of the known viruses in one or more of their hosts (Hull, 2002).

Seed transmission plays a pivotal role in the spread and survival of a number of important plant viral diseases. Infected seed is probably the most important source of viruses and sub-viral pathogens in commercial plantings. In fields, the seedlings raised from the randomly dispersed infected seeds serve as initial sources of virus inoculum or foci of infection from which secondary spread occurs within and outside the field by suitable vectors. Besides being a source of inoculum, the seed also helps in perpetuation of the virus over long periods (Chalam and Khetarpal, 2008).

Pathogenic infections can infect seeds internally and destroy the endosperm and the embryo or contaminate the seeds and affect seedling development. Seed borne pathogens primarily cause disease of seeds and have been involved in seed rots during germination and seedling mortality leading to poor crop stand reduction in plant growth and productivity of crops (Akranuchat *et al.,* 2007).Apart from this, infected seeds act as a vehicle in carrying pathogens to uninfected areas within a country and from one country to the other (Waller, 2002; Albrechtsen, 2006). Seed sterilization is therefore an important process that provides insurance against seed-borne as well as soil-borne plant pathogens and insects (Gwary *et al.,* 2007).

In the case of plant viral diseases, different chemical and physical treatments have been reported to eradicate or significantly reduce the incidence of a number of viruses without affecting seed quality. In an experiment by Rast and Stijger (1987), the sterilization of pepper seed infected with Capsicum Mosaic Virus (CaMV) by immersion in 100 g/1 $Na_3 P0_4$ solution was compared with dry heat treatment at 76°C. The virus content of the seed varied with the CaMV strains used to infect the pepper cultivars and the time of harvest of seeds from infected plants.Some other reports indicate that although some seed companies currently utilize pre-treatments seeds, the details of these seed treatment protocols are however proprietary (Córdoba-Sellés *et al.,* 2007).

The objectives of the study were to test the efficacy of Sodium hypochlorite and Ethanol as seed sterilants on cowpea variety 96D-GlO infected with Cowpea Mottle Virus and assess the effect on growth and yield of the crop. It will also quantify the concentration of the sterilant that most significantly reduced severity of the viral disease.

MATERIALS AND METHODS

Source of seed variety and virus inoculum

The potted experiment was carried out in the screenhouse of the Crop Protection Department, Faculty of Agriculture, University of Ilorin – Nigeria. Cowpea variety 96D-GLOand Cowpea Mottle Virus (CPMV) inoculum wereobtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Soil sterilization procedure

The soil used was steam sterilization to a temperature of 80°C for 30minutes, followed by an 8-min resting period resulted in 100% kill of all weeds and soil pathogens (van Loenen *et al.,* 2003). The soil was then potted into plastic perforated buckets of 10 litre capacity prior to the sowing of the treated inoculated seeds.

Virus inoculation and seed Sterilization

The cowpea seeds were firstly mechanical inoculated with Cowpea Mottle virus (CPMV) by slightly dusting the seeds with carborundum to act as slight abrasive agent. This was followed bygently rubbing the seeds with the viral inoculum using cotton wool at the rate of 5ml inoculum per seed. The seeds were thereafter sun dried for 1 hour before sowing into plastic buckets at the rate of 6 seeds per pot (Olaitan *et al*., 2009).

The sterilants were at the following three concentrations: Sodium hypochlorite (NaClO) 0.5%, 0.75%, 1.0%; Ethanol (C_2H_6O) 75%, 85%, 95% and Distilled water served as control for the experiment.

Each treatment was replicated five times and the pots werearranged on raised platforms while irrigation was donetwice in three days until the emergence of flowering.

Data collection and Statistical analysis

Data collected from the 3rd to 10th week after planting (WAP)were: plant height (cm), number of leaves per plant, number of leaves showing characteristic virus disease symptoms (such as mild to severe chlorotic mottling, mosaic patterns and distortion), the percentage virus disease severity measured by the number of diseased leaves relative to the total number of leaves on any given plant and weight of pods and seeds per plant at 80 days after planting.

All collected data were subjected to analysis of variance (ANOVA) using the Statistical Package for the Social Sciences SPSS version 21and the means if significant, separated using the New Duncan Multiple Range Test at 5% level of probability.

RESULTS

Effect on virus severity

Table 1 shows the effects of the treatments on the severity of virus on cowpea seedlings at different times after planting. The results showed that there were significant ($P \geq 0{\cdot}05$) differences between treatments in their efficacy to limit virus severity. Disease initiation for all treatments started at the 4th WAP and the significantly ($P \geq 0{\cdot}05$) lowest percentage virus disease severity were in plants sterilized with Ethanol. Further scrutiny of the values indicated that at 4th,5th, 6th, 7th, 8th, 9thand 10thWAP, sterilization of cowpea seeds with 95% ethanol provided the best protection with the significantly ($P \geq 0{\cdot}05$) lowest disease severity values of 1.4%, 3.7%, 6.73%, 15.43%, 16.83% and 17.63% respectively. The next most effective disinfectant in ameliorating virus disease severity after Ethanol was Sodium hypochlorite. It was also observed (Table 1), that NaClO was significantly ($P \geq 0{\cdot}05$) more effective than distilled water especially at a concentration of 1%. At the 10th WAP, sterilizing cowpea with NaClO at 1% concentration resulted insignificantly ($P \geq 0{\cdot}05$) lower virus disease severity (26.17%) compared with distilled water (39.77%).

Table 1: Effect of treatment on disease severity

	Weeks After Planting (WAP)						
Treatment	**4wk**	**5wk**	**6wk**	**7wk**	**8wk**	**9wk**	**10wk**
NaClO 0.5%	9.80^{a}	13.53^{b}	16.03^{b}	24.37^{b}	29.20^{b}	30.73^{b}	32.60^{b}
NaClO 0.75%	6.23^{c}	11.67^{c}	15.27^{b}	22.33^{c}	26.57^{c}	29.80^{b}	31.40^{c}
NaClO 1%	3.70^{d}	9.27^{d}	12.57^{c}	18.13^{d}	23.27^{d}	24.73^{c}	26.17^{d}
Ethanol 75%	3.40^{d}	6.57^{e}	11.67^{c}	18.57^{d}	22.90^{d}	24.27^{c}	24.93^{e}
Ethanol 85%	2.77^{e}	5.17^{f}	8.90^{d}	14.20^{e}	18.37^{e}	20.40^{d}	21.53^{f}
Ethanol 95%	1.40^{f}	3.70^{g}	6.73^{e}	11.47^{f}	15.43^{f}	16.83^{f}	17.63^{g}
Control	8.43^{b}	15.50^{a}	19.80^{a}	26.27^{a}	32.37^{a}	37.37^{a}	39.77^{a}
S.E.M	0.649	0.922	0.925	1.118	1.233	1.433	1.545

Means within a column followed by the same letter(s) are not significantly different using the New Duncan Multiple Range Test at $P \geq 0.05$. NS signifies not significant.

Effect on plant height

The effect of treatments on plant height from 4th to 10th WAP is shown in Table 2. The effect ofthe treatments on plant height was apparent from the 5thto 10th WAP. The tallest plants at the 5th WAP were in the treatment with 95% Ethanol (30.27cm), 85% Ethanol (30.03cm), and 1% NaOCl (29.30cm) and these values were not significantly different ($P \geq 0.05$) from Ethanol 75% (27.77cm), NaOCl 0.5% (27.63cm) and NaOCl 0.75% (27.33). The significantly shortest plants were the control (25.73cm). The same trend was observed from the 6th to 10th WAP, whereby the significantly tallest plants were in the treatments with Ethanol 95% (39.57 – 59.13cm) and shortest plants in the

control with values ranging from 29.47 to 40.73cm.

Table 2: Effect of treatment on plant height (cm)

	Weeks after Planting							
Treatment	**3wk**	**4wk**	**5wk**	**6wk**	**7wk**	**8wk**	**9wk**	**10wk**
NaClO 0.5%	22.43	25.37	27.63^{ab}	30.13^{e}	33.63^{e}	39.80^{e}	42.37^{d}	43.90^{e}
NaClO 0.75%	22.30	24.13	27.33^{ab}	32.23^{d}	35.53^{d}	41.90^{d}	44.07^{d}	45.93^{d}
NaClO 1%	21.80	24.37	29.30^{a}	35.97^{bc}	42.37^{c}	48.40^{c}	50.40^{c}	52.97^{c}
Ethanol 75%	21.17	23.83	27.77^{ab}	35.23^{c}	41.67^{c}	47.13^{c}	48.80^{c}	51.43^{c}
Ethanol 85%	22.57	24.50	30.03^{a}	37.33^{b}	44.67^{b}	51.53^{b}	53.43^{b}	55.17^{b}
Ethanol 95%	20.30	22.43	30.27^{a}	39.57^{a}	48.07^{a}	55.57^{a}	57.57^{a}	59.13^{a}
Control	21.87	23.63	25.73^{b}	29.47^{e}	32.53^{e}	36.23^{f}	38.87^{e}	40.73^{e}
S.E.M	0.368	0.368	0.453	0.808	1.237	1.427	1.371	1.378

Means within a column followed by the same letter(s) are not significantly different using the New Duncan Multiple Range Test at P≥0.05.

Effect on number of leaves per plant

The results of the effect of the treatments on mean number of leaves per plant (Table 3) revealed that there were significant differences among the treatment means from 6th to 10th WAP. The seeds treated with Ethanol at 95% produced the significantly highest average number of leaves per plant (58.17 to 72.30) for the duration of the experiment.

Table 3: Effect of treatment on number of leaves per plant

	Weeks after Planting (WAP)							
Treatment	**3wk**	**4wk**	**5wk**	**6wk**	**7wk**	**8wk**	**9wk**	**10wk**
NaClO 0.5%	21.70	32.70	43.87	48.63^{c}	54.03^{c}	59.33^{c}	62.30^{c}	63.33^{c}
NaClO 0.75%	20.73	31.80	44.03	49.27^{c}	54.17^{c}	59.30^{c}	62.27^{c}	63.63^{c}
NaClO 1%	21.27	30.20	44.77	50.10^{c}	56.50^{bc}	62.70^{b}	65.70^{b}	66.87^{b}
Ethanol 75%	20.43	31.17	44.80	49.70^{c}	56.10^{bc}	62.63^{b}	65.50^{b}	66.53^{c}
Ethanol 85%	21.13	31.87	44.30	52.80^{b}	59.27^{b}	61.67^{b}	64.93^{b}	66.07^{b}
Ethanol 95%	21.03	32.60	44.77	58.17^{a}	65.40^{a}	67.30^{a}	71.47^{a}	72.30^{a}
Control	22.60 NS	31.57 NS	44.17 NS	49.17^{c}	55.03^{c}	61.30^{b}	64.57^{b}	67.30^{b}

NS = not significant

Means within a column followed by the same letter(s) are not significantly different using the New Duncan Multiple Range Test at P ≥0.05.

Effect on yield parameters

The effect of the treatments on yield parameters as shown in Table 4 indicated that the significantly highest number of pods (37.17g) and seed weights (36.07g) were obtained in the treatment with 95% Ethanol compared to an average number of 8.63 pods per plant and 7.7g seed weight obtained in the control treatment.

Table 4: Effect of treatment on yield

Treatment	Yield Parameters (g) Wt. of pods/plant	Wt. of seeds/plant
NaClO 0.5%	12.77[d]	11.47[d]
NaClO 0.75%	14.73[d]	13.37[d]
NaClO 1%	20.47[c]	19.00[c]
Ethanol 75%	20.40[c]	18.97[c]
Ethanol 85%	25.30[b]	23.47[b]
Ethanol 95%	37.17[a]	36.07[a]
Control	8.63[e]	7.70[e]
S.E.M	1.965	1.953

Means within a column followed by the same letter(s) are not significantly different using the New Duncan Multiple Range Test at P $\geq$0.05.

Discussion

The present study affirms the efficacy of Ethanol and Sodium hypochlorite in ameliorating virus disease severity in cowpea. The elucidation of the exact mechanism of action of disinfectants against plant viruses could be difficult because viruses are template nucleic acid molecules embedded in lipo-protein bilayer. It can be assumed therefore that the ability of the sterilants to ameliorate virus severity in cowpea variety 96D-GLO was due to the concentration of hydrogen (H^+) and hydroxyl (OH^-) ions in both Ethanol and Sodium hypochlorite. The H^+ ions most probably destroyed the amino – acid bond in the nucleic acids of the virus thereby modifying the cytoplasmic pH, thus leading to the precipitation and dissolution of the virus protein.

The OH^- ions could also have caused the saponification of some lipids in the enveloping membrane, thus leading to the destruction of the superficial structure and subsequent hydrolysis of the nucleotides of the virus genome. This view is also shared by Dauphin and Darbord (1988) on the actions of some sterilants against plant virus viability. Kuyyakanond and Quesnel (1992) had earlier postulated that the presence of lipids in a virus is uniformly associated with a high degree of susceptibility to disinfectants and the absence of lipid and small size are associated with resistance to lipophilic chemical agents and sterilants. The report is consistent with Hu *et al.* (1994) which also found that undiluted skim milk and commercial bleach used as seed sterilantsat 10% or 20% concentration inactivated Cymbidium Mosaic Virus (CyMV) and Odontoglossum Ringspot Virus (ORSV) on a local lesion host.

The observation of action of the sterilants on height and number of leaves of the plants revealed that seeds treated with either Ethanol or Sodium hypochlorite had significantly taller plants and produced more leaves. This probably suggests that both sterilants improved plant growth by increasing germination time. This finding is in agreement with submissions by Ho *et al.* (1995) and Pernezny *et al.* 2002. They also opined that Sodium hypochlorite, Phenolic and Formaldehyde compounds accelerated germination and improved the rate of plant growth in all the cases considered. Similar results were also reported by Sen *et al.* (2013).

In most instances disease is logically related to yield and disease severity is negatively correlated with yield. This implies that the growing and production status of plants is affected by their level of disease susceptibility. This study verified that cowpea seed sterilization with Ethanol and Sodium hypochlorite cause reduction in disease severity and eventual higher plant yields. The effectiveness of the

disinfectants to ameliorate disease severity is a considerable aspect of yield verification. This can be deduced as the basis for the higher pod and seed weights obtained in the study. This observation is in agreement with those made by Jan *et al.* (2013), who in a study reported that surface sterilization produced higher yields of field grown strawberry explants intended for in vitro culture.

Conclusion

The study has presented at first glance, the effect of Sodium hypochlorite and Ethanol at different concentrations as seed sterilants on cowpea seeds mechanically infected with Cowpea Mottle Virus. The study established that the sterilants were the main reason for the reduction in viral disease on the cowpea plants. This reduction subsequently resulted to improvements in the growth and yield capacity of the crop. The effect was however more assertive in surface sterilization with Ethanol (95%). The outcome of this study shows greater prospect in the application of Ethanol (95%) as seed sterilant to control virus diseases in cowpea.

Conflict of interest

Author declares no conflict of interest.

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