Journal of Agriculture and Sustainability

ISSN 2201-4357

Volume 7, Number 1, 2015, 87-98

Reduction of Seed-Borne Fungi of the Genus *Aspergillus* Associated with Egusi Melon *Colocynthis citrullus* (L.) Seeds Using Chlorine Disinfectants – Implications on Seed Germination

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Abstract: A laboratory trial was conducted in 2014 at Michael OKPARA University of Agriculture, Umudike, Abia State of Nigeria to determine the effects of three chlorine disinfectants: potassium chloride, mercuric chloride and sodium hypochlorite on *Aspergilus. niger, A. flavus, A. fumigatus* and *A. granulosis* on seeds of *Colocynthis citrullus* (egusi melon). 400 seeds were pretreated with different disinfectant at different concentrations (0.1,1.0 and 2.0%) with distilled water as the control and at different time regimes (1, 3, 5 and 7 minutes). The surface sterilized seeds were cultured and incubated at 25±2^{0}C for 7days. After which the seeds were examined for fungi growth and identification. Data obtained showed seed treatment with mercuric chloride was most effective in the reduction of *A. flavus* in *C. citrullus* seeds. Treatment of *C. citrullus* seeds with potassium chloride and sodium hypochlorite resulted in significant reduction in percentage recovery of *A. flavus* when compared with the control (distilled water). However, increase in concentrations of these two chloride disinfectants resulted reduction in percentage recovery of the pathogen but were more effective as surface disinfectants for *C. citrullus* seeds at low concentrations. From this study therefore to protect the seeds from surface infection and contamination by *Aspergillus* spp and other micro flora during storage and to maintain their viability status and also considering the toxic implications and environmental hazards of mercuric chloride, sodium hypochlorite becomes a preferred seed disinfectant chemical.

Keywords: *Colocynthis citrullus*, *Aspergillus,* Chlorin**e,** disinfectants and hypochlorite.

INTRODUCTION

Egusi melon (*Colocynthis citrullus* L.) is an important member of the Cucurbitaceae family as it is a source of dietary protein especially in West Africa. A major challenge facing the production of *C. citrullus* is the incidence of seed-borne fungal pathogens which cause deterioration of seed by seed necrosis, discolouration, deformation and seed rot during storage in between planting seasons (Chuku *et al*., 2010). This causes a progressive hike in price of *C. citrullus* seeds between the harvest season and the next planting season. Seed-borne fungi also reduce germination (Nagaraja and Krishnappa, 2009; Chiejina, 2006; Chuku *et al*., 2010), and may significantly impact on the yield. Chemical disinfectants, especially chloride disinfectants such as sodium hypochlorite and mercury chloride have been shown to be effective in the control of seed-borne fungal pathogens. Sitara and Akhter, (2007) evaluated the disinfectant effect of sodium hypochlorite on fungal pathogens of *Zea mays* seed. They reported that 10% sodium hypochlorite eliminated *Phoma spp* and *Chaetomium spp* while reducing infection by *Aspergillus niger, A. flavus, A. fumigatus, A. candidatus, Rhizopus spp* and *Nigrospora spp.* Barbarosa-Martinez *et al* (2012) reported the effectiveness of chlorine at 360mgL^{-1} on *Colletotrichum gleosporoides, Pestalotiopsis mangiferae, Fusarium oxysporum* and *Lasiodiploidia theobromae* isolated from mango fruit. Nwangburuka *et al* (2013) showed that sodium hypochlorite had significant antifungal effect on fungal pathogens of *Abelmoschus esculenta* which include *Fusarium, Alternaria* and *Rhizopus spp.*

Although chloride disinfectants are effective in reducing or eliminating seed-borne fungal pathogens, there is valid concern for their effects on the seed biochemistry and viability. This is because stored seed is required for future cultivation of the crop to ensure continuity. Reports by Jagatheeswari and Ranganathan (2012) based on studies on *Vigna radiata* indicate that treatment of seeds with mercuric chloride could lead to a drastic reduction in length and weight of shoots and roots, chlorophyll concentration, protein content, starch content and seed germination. This was further stressed by

Abraham *et al* (2012) and Abraham and Damodharan (2012) who indicated that increasing concentrations of mercuric chloride reduced seed germination, total chlorophyll and protein content of *Arachis hypogeae* seeds with respect to control in a dose-dependent regime.

The effect of sodium hypochlorite on seed germination is unclear. Data from the report by Nwangburuka *et al* (2013) indicates that different concentrations of sodium hypochlorite gave rise to different effects. For example, 1% sodium hypochlorite increases the germination although not significantly, while 2% and 3% drastically reduced the germination of *A. esculenta* seeds. Reports by Khah and Passam (1992) is unclear about the effect of sodium hypochlorite concentration on seed germination. Washing with distilled water increased the germination of seeds treated with 1% sodium hypochlorite although not significantly, but reduced those treated with 3% sodium hypochlorite. Similarly, only short duration, high temperature incubation of treated seeds had a positive effect on germination. Sitara and Akhter (2007) reported that concentration of sodium hypochlorite had no significant effect on the germination of maize seeds.

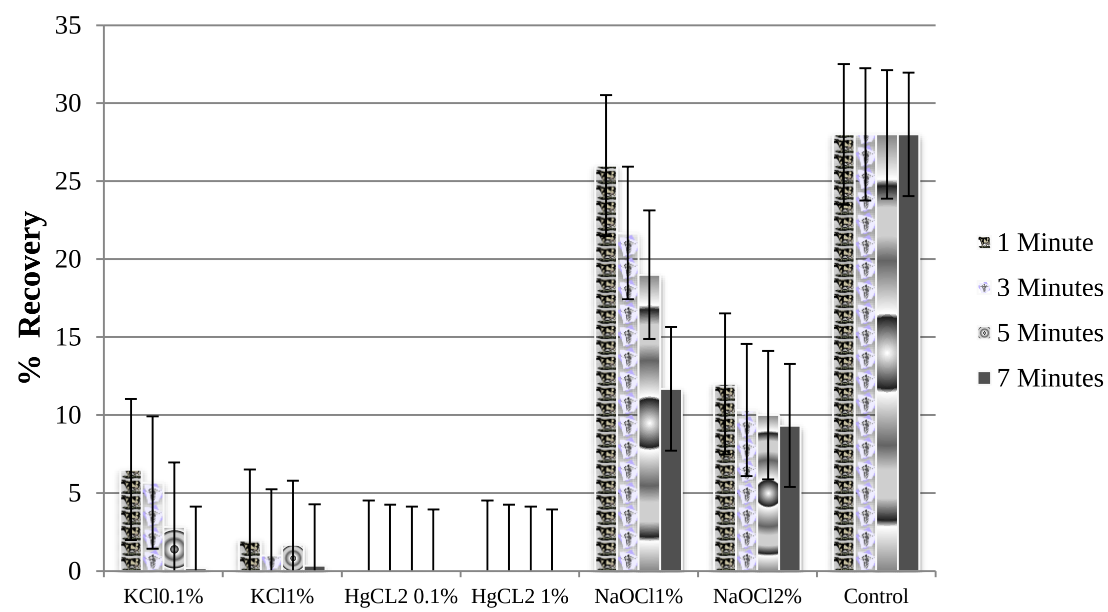
In this study, the effects of three chlorine disinfectants; potassium chloride, mercuric chloride and sodium hypochlorite on *A. niger, A. flavus, A. fumigatus* and *A. granulosis* were evaluated *in vivo* at different time durations and the effects of the disinfectants on germination of *C. citrullus* seeds determined.

MATERIALS AND METHODS

The egusi melon (*C. citrullus*) seeds ('Serewe' cultivar) were sourced from farmers in the major egusi growing areas of the South-Eastern region of Nigeria. 400 *C. citrullus* seeds were used per disinfectant at different concentrations (mecuric chloride (HgCl), potassium chloride (KCl) and sodium hypochlorite (NaOCl) at 0.1,1.0 and 2.0% while distilled water served as control) in 500ml beakers at and at different time regimes: 1, 3, 5 and 7 minutes. Only sodium hypochlorite had pretreatment at 1and 2.0% while others were at 0.1 and 1.0% distilled water served as the control. At the end of each pre-treatment period, the seeds were rinsed in three changes of distilled water. The surface sterilized seeds were placed on three blotters moistened with sterile distilled water in 9cm Petri dishes (10seeds/dish). Treated seeds were cultured and incubated at 25±2^{0}C for 7days. At the end of the incubation period, the seeds were examined for fungi growth and were identified using fungi identification manual modified by Umechuruba and Elenwo (1996), Barnett and Hunter (1999), Alexopoulous *et al* (2002). Also percentage seed germination was also determined. A seed was considered germinated with the emergence of a radicle.

RESULTS AND DISCUSSION

Fig. 1: Effects of chloride disinfectant concentration and contact time on the recovery of *A. flavus* from *C. citrullus* (egusi melon) seeds



The result in fig.1 shows that seed treatment with mercuric chloride was most effective in the control of *A. flavus* in *C. citrullus* seeds as both concentrations tested yielded seeds from which no *A. flavus* could be recovered by the test method, regardless of the contact time. This suggests that mercuric chloride is very potent in the control of *A. flavus.* Treatment of *C. citrullus* seeds with potassium chloride and sodium hypochlorite resulted in significant reduction in percentage recovery of *A. flavus* from treated seeds when compared with the control (distilled water), however, at higher concentrations of these two chloride gave lower values of percentage recovery. Potassium chloride was significantly more effective than sodium hypochlorite in the control of *A. flavus.* 0.1% sodium hypochlorite treatment at 1, 2 and 3 minute regimes, though recovery of *A. flavus* was not significantly different from the control values. Also, it is important to note that increase in contact time between the *C. citrullus* seeds and disinfectants resulted in lower recovery of *A. flavus*. This implies that contact time is a major factor in decontamination of *C. citrullus* seeds using chloride disinfectants.

Fig. 2: Effects of chloride disinfectant concentration and contact time on the recovery of *A. fumigatus* from *C. citrullus* (egusi melon) seeds

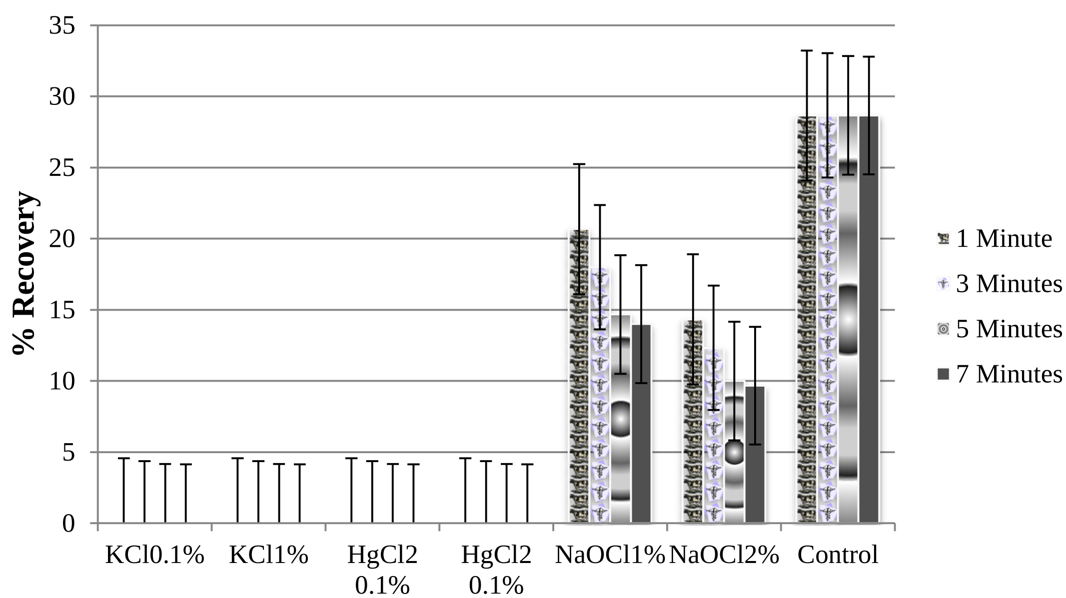
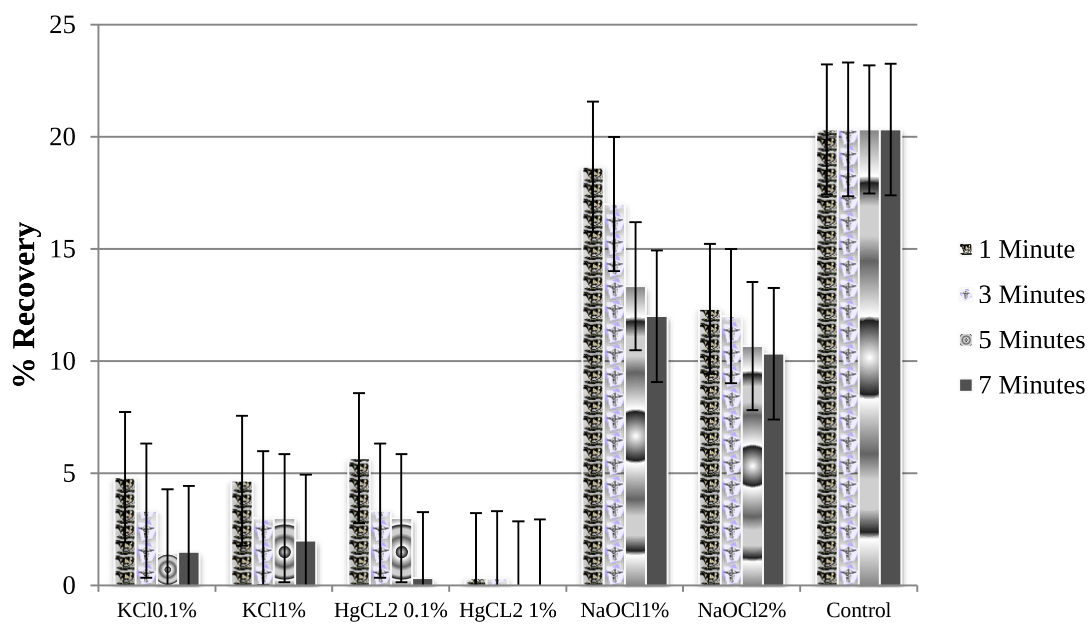


Fig. 2 shows that treatment of *C. citrullus* seeds with potassium chloride and mercuric chloride had the maximum effect, regardless of contact time, as they produced seeds from which no *A. fumigatus* could be recovered. Sodium hypochlorite showed significant effect, when compared to control values especially at 2%. From the data 1% sodium hypochlorite gave significantly different percentage recovery at all contact times tested except for 1 minute. This suggests that longer contact time is required for effective action of sodium hypochlorite on *A. fumigatus* in *C. citrullus* seeds, as can be seen in 2% sodium hypochlorite-treated seeds.

Fig. 3: Effects of chloride disinfectant concentration and contact timeon the recovery of *A. granulosis* from *C. citrullus* (egusi melon) seeds



Out of all the treatment *Aspergillus* species tested, only *A. granulosis* gave the highest resistance to disinfectant action. This is shown in fig. 3 as the highest concentration of mercuric chloride that was able to completely eliminate *A. granulosis* from treated *C. citrullus* seeds. It is also important to note that 0.1% mercuric chloride also gave maximum elimination of *A. granulosis* at the highest contact time tested. This indicates that similar results may be obtained by using lower concentrations of disinfectant at higher contact time duration. This is further stressed by the observation that that treatment with 1% sodium hypochlorite for 7 minutes gave lower percentage recovery of *A. granulosis* than those with 2% sodium hypochlorite at 1 and 3 minutes duration. The use of lower concentrations of disinfectants, regardless of contact time could result in cost savings for rural farmers as less disinfectant is required.

There is no significant difference between recovery of *A. granulosis* from *C. citrullus* seeds treated with both concentrations of potassium chloride and 2% mercuric chloride at contact times of 1 and 5 minutes intervals. Also, significant differences in percentage recovery of *A. granulosis* was observed between samples treated with sodium hypochlorite, regardless of concentration and treatment time tested and other chloride disinfectants tested. Furthermore, treatment of *C. citrullus* seeds with 1% sodium hypochlorite for 1 and 3 minutes did not reduce the recovery of *A. granulosis* significantly when compared with the control.

Fig. 4: Effects of chloride disinfectant concentration and contact time on the recovery of A. niger from *C. citrullus* (egusi melon) seeds

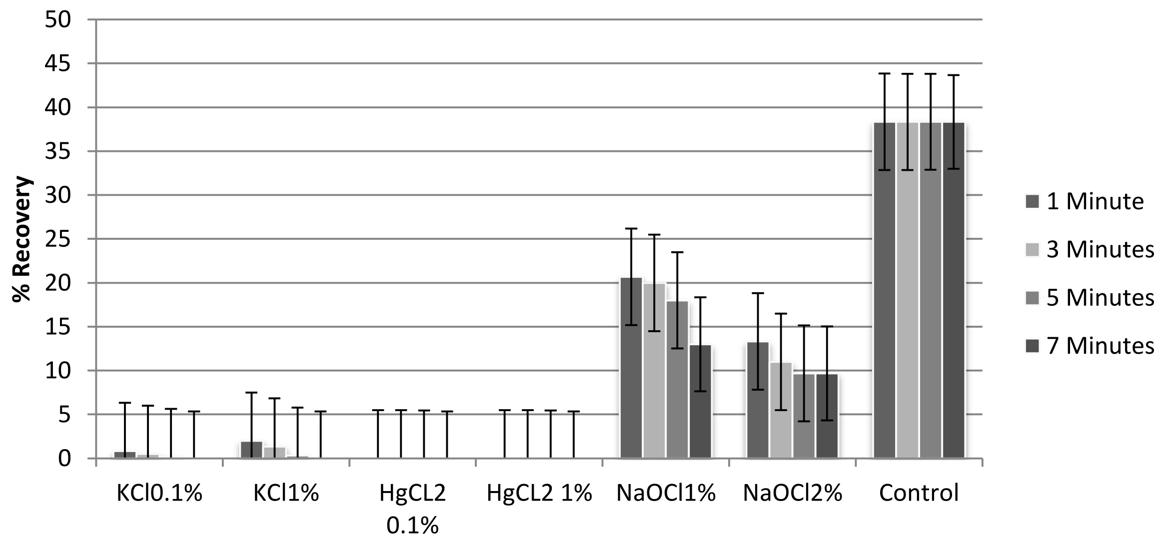


Fig 4 shows that mercuric chloride is effective in eliminating *A. niger* from *C. citrullus* seeds at all concentrations and contact time duration tested. Similarly, potassium chloride was also found to be very effective. However, *A. niger* was recovered at the lowest contact time tested (1 minute), even though this is not significantly different from results obtained from mercuric chloride. Percentage recovery of *A. niger* from seeds treated with sodium hypochlorite show significant difference from both control values and those observed from treatment with mercuric chloride and potassium chloride, regardless of concentration and contact time. Percentage recovery of *A. niger* from *C. citrullus* seeds treated with sodium hypochlorite reduced with increase in contact time. Seed treatment with mercuric chloride was most effective in the control of *A. flavus* in *C. citrullus* seeds as both concentrations tested yielded seeds from which no *A. flavus* could be recovered by the test method, regardless of the contact time. This suggests that mercuric chloride is very potent in the control of *A. flavus.* Treatment of *C. citrullus* seeds with potassium chloride and sodium hypochlorite resulted in significant reduction in percentage recovery of *A. flavus* from treated seeds when compared with the control. However, higher concentrations of these two chloride disinfectants gave lower values for percentage recovery.

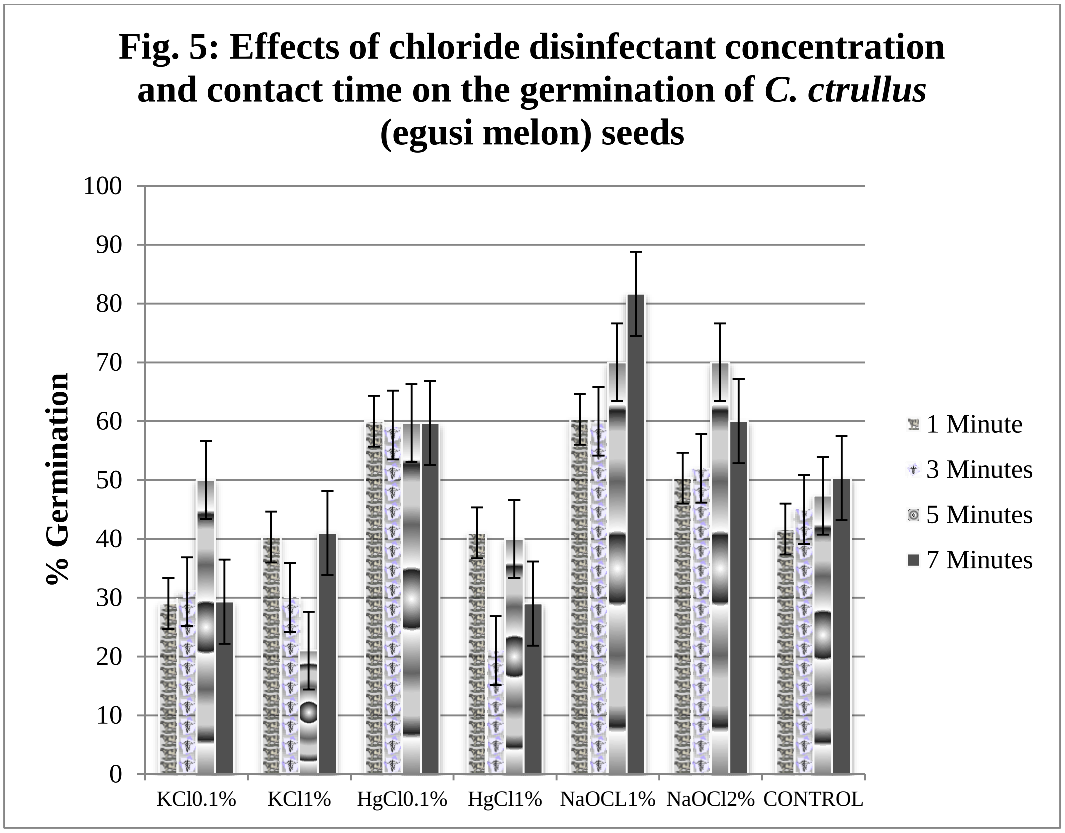


Fig. 5 shows the impact of treatment of *C. citrullus* seeds on germination. From the chart treatment of *C. citrullus* seeds with 0.1% sodium hypochlorite gave the highest germination, which was significantly (P=0.05) higher than the control samples at 5 and 7 minutes. Germination% increased with increase in contact time with the highest germination obtained from seeds treated with 0.1% sodium hypochlorite for 7 minutes. Seed pre-treated with 2% sodium hypochlorite showed increase in germination with respect to increase in contact time. Maximum germination was observed at 5 minutes of contact time. Further contact resulted in reduced germination.

It is important to note that treatment of *C. citrullus* seeds with 0.1% mercuric chloride significantly increased seed germination while 1% mercuric chloride reduced germination. This is in agreement with the reports of Abraham *et al* (2012) and Jagatheeswari and Ranganathan (2012) on the inhibitory action of high concentrations of mercuric chloride on seed germination. Furthermore, the data showed that there is no significant differences in their effect on seed-borne nature of *Aspergillus spp*. Germination was fairly constant in samples treated with 0.1% mercuric chloride regardless of the contact time.

Potassium chloride treatment generally resulted in a reduction in germination of *C. citrullus* seeds, this was significantly (P=0.05) from seeds treated with 0.1% potassium chloride for 1 minute and seeds treated with 1% potassium chloride for 3 and 5 minutes. Only seeds treated with 0.1% potassium chloride for 5 minutes showed increased germination percentage. However, these results did not follow a particular trend.

For all fungi tested, sodium hypochlorite had the lowest effect, even at double the concentration tested. This finding agrees with Sitara and Akhter (2007), who reported that sodium hypochlorite was not effective in the recovery of *A. flavus*. Khah and Passam (1992) in their work also found that higher concentrations of sodium hypochlorite reduced germination of sweet pepper. This also occurs in *C. citrullus* (egusi melon).

From the data obtained in this study, mecuric chloride, potassium chloride and sodium hypochlorite should be considered as effective surface disinfectants for *C. citrullus* seeds especially at low concentrations, so as to protect the seeds from the attack of *Aspergillus* spp during storage and maintain their viability. However, there is still need for further research into the effect of these and other chemicals as disinfectants and for the control of other seed-borne fungi and the effect of long term storage on control of seed-borne fungi by disinfectants, considering toxic implications and environmental safety.

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