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THE INTERNATIONAL CONFERENCE ON BIOTECHNOLOGY 2024

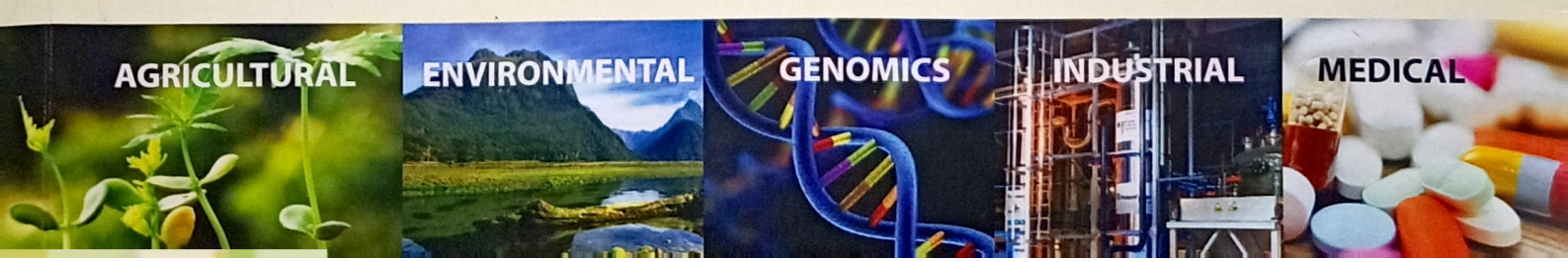
15th - 19th JULY, 2024

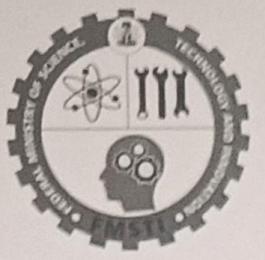
NAF Conference Center Abuja

Theme

"BIOTECHNOLOGY AS AN ENGINE FOR ECONOMIC GROWTH"

CONFERENCE PROCEEDINGS







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INTERNATIONAL CONFERENCE OF BIOTECHNOLOGY, 2024 (ICoB24)
CONFERENCE PROCEEDINGS

Published By

NATIONAL BIOTECHNOLOGY RESEARCH AND DEVELOPMENT AGENCY (NBRDA)
ABUJA, NIGERIA

JULY 2024

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Table 1 The effect of different concentrations of $HgCl_2$ and time on sterilization of explants for the survival of the Neem tree.

Traits	S.O.V	Df	MS
Browning	$HgCl_2$	4	0.152***
	Time	3	0.012^{ns}
	$HgCl_2$×Time	12	0.24^{ns}
	Error	40	0.039
Fungal contamination	$HgCl_2$	4	0.816***
	Time	3	0.024^{ns}
	$HgCl_2$×Time	12	0.084^{ns}
	Error	40	0.061
Survival	$HgCl_2$	4	0.726***
	Time	3	0.013^{ns}
	$HgCl_2$×Time	12	0.095^{ns}
	Error	40	0.067

Values with ***, *, ns means differ significantly at ($p < 0.01$), ($p < 0.05$), No significant difference at ($p < 0.05$) respectively.

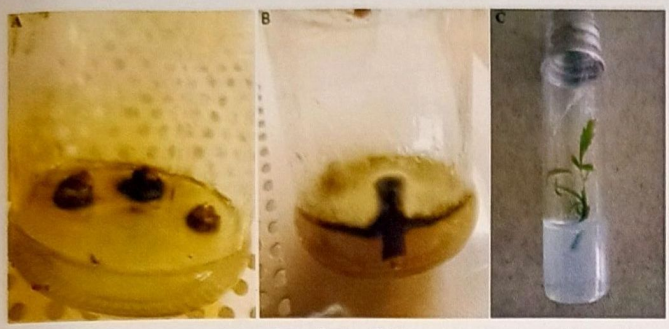

Figure 2 (a). Browning of explants (b) Explants contaminated by fungi (c). Healthy Neem explants

Likewise, time and the interaction between time and $HgCl_2$ showed no significant difference in browning, fungal contamination, and survival percentage of the explant (Table 1). To conclude, the explants were not at all contaminated by bacteria. The most difficult problem is obtaining survived explants of neem in its tissue culture. The explants were mainly contaminated by fungus and rarely by bacteria, as stated by(Chaturvedi et al., 2004) and confirmed in the present study. This result shows less browning in all the treatments, except 0.2% $HgCl_2$ which shows the highest browning percentage due to the accumulation and activity of the phenolic compound, and also a high concentration of $HgCl_2$ may be toxic to the explants as obtained by(Hashim et al., 2021).

Furthermore, the fungal attack was also lesser at the 0.15% and 0.2% $HgCl_2$concentration, indicating the effectiveness of the HgCl2 in sterilizing the neem tree. This result is in agreement with the findings of(Chaturvedi et al., 2004) and(Bello et al., 2022) for direct somatic embryogenesis. The studies of(Rafiq and Dahot, 2010) and (Houllou et al., 2015) were all contrary to the present findings because of the different parts of the plants they used, usually immature fruits, leaflets, and flower petals, or due to the genetic content of the plant used, most of them also carried out indirect somatic embryogenesis, which is also contrary to our research.

CONCLUSION

Based on the result obtained, it can be concluded that the neem tree can be established for plant tissue culture by using $HgCl_2$ as a sterilizing agent for 10 to 13 minutes at a 0.15% concentration. A higher concentration of 0.2% causes a lot of browning. It can also be deduced that Neem is rarely contaminated by bacteria and mostly by fungi at all concentrations of $HgCl_2$.

Recommendations

Different sterilization times should also be investigated to find the exact sterilization time for the establishment of the plant.

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Germination Performance of Cowpea (*Vigna unguiculata*) Seeds Treated with Entomopathogenic Isolates

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INTRODUCTION

Pesticide treatment of stored seed against pests should not come at the expense of seed quality including the seed germination viability ((Dan *et al.* 2012). Pesticide treatments may have the potential to affect seed germination and overall viability depending on the type of pesticide used, the concentration applied, and the specific characteristics of the seeds themselves (Araújo *et al.*, 2023). Synthetic chemicals have been persistently used in agriculture for field and stored pests, especially in developing countries which negatively affects the growth and physiological parameters of the

crop including the germination performance of the seed (Maldani *et al*. 2021). These synthetic chemical pesticides not only affect crop growth and physiology but also have shortcomings, especially in the food safety of crops when used as storage pesticides, as the produce usually contains high levels of chemical pesticides considered dangerous to human health (Nwosu and John, 2022). Several studies have also shown that it pollutes the environment, contributes to climate change, and affects non-target organisms when also applied in fields.

The use of biological control measures as an alternative to synthetic chemical pesticides is increasingly encouraged owing to the problem of toxicity the synthetic chemicals impact on crops. Among the most globally considered biological control measures for agricultural pests is the use of entomopathogenic microorganisms. These entomopathogens are naturally occurring microorganisms with insecticidal properties against several agricultural pests including Lepidoptera, Diptera, Coleoptera, and Hymenoptera among others. However, limited attention is paid to the impact of these entomopathogens used as pesticides on the germination performance of seeds. In this study, the germination viability of stored cowpea seeds treated with isolates of entomopathogens (*Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii* and *Bacillus thuringensis*) usually considered suitable candidates in research efforts towards developing microbial-based options for agricultural pest control was investigated.

METHODOLOGY

The pure microbial isolates were subjected to liquid fermentation technology using sucrose water as a substrate to maximize the microbial load. The isolates of *Beauveria bassiana*, *Verticillium lecani*, *Metarhizium anisopliae* and *Bacillus thuringensis* obtained through liquid fermentation with a microbial load of 2.56×10^4 CFU/ml, 4.44×10^6 CFU/ml, 2.15×10^5 CFU/ml and 3.66×10^6 CFU/ml were also at 20ml inoculated each into 50gms of sterilized talc powder, sealed appropriately and stored at room temperature as solid media for one month and microbial load determined. The germination performance of cowpea seeds treated with microbial isolates was performed as described by Behluli *et al.* (2016). Five (5) ml of the liquid-fermented isolates of *Beauveria bassiana*, *Verticillium lecani*, *Metarhizium anisopliae* and *Bacillus thuringensis* respectively were separately sprayed in air-tight containers containing 100gms of cowpea seeds. Also, 5g of solid media of the isolates of *Beauveria bassiana*, *Verticillium lecani*, *Metarhizium anisopliae* and *Bacillus thuringensis* respectively were applied in air-tight containers containing 100gms of the cowpea seeds.

The germination viability of the cowpea seeds treated with the liquid and solid media of the microbial isolates and stored for ten (10) weeks was determined using the standard germination test adopting the seed germination vigour (SGV) and the seed germination ability (SGA) as key parameters. The SGV was calculated based on the germination percentage on half of the complete days of the experiment while the SGA was calculated based on the percentage of seed germination on the final day of the testing. Germination counts were taken from 1 to 6 days after sowing. Seeds were considered germinated when the tip of the radicle had grown free from the seed coat, otherwise known as radical emergence (El Balla *et al.,* 2011).

RESULTS

The study revealed that the cowpea seed treatment with the microbial isolates in their liquid and solid media showed no significant impact when compared with control on both SGV and SGA at the end of 1st and 5th-week storage post-treatment. However, a significant effect was seen at the end of 10th-week storage post-treatment with the liquid medium of *Verticillium lecanii* showing better germination performance (SGV = 58.33±1.67 and SGA = 71.67±3.33) among other isolates in a liquid medium when compared with the control (SGV= 68.33±1.67 and SGA = 80.00±2.89) and the isolate of *Bacillus thuringensis* in its solid medium revealing better germination performance (SGV= 58.33±3.33 and SGA= 71.67±1.67) among other isolates in a solid medium when compared with the control (SGV= 68.33±1.67 and SGA= 80.00±2.89). The comparative analysis of the treatment with the microbial isolates in its liquid and solid media for SGV and SGA showed no significant difference with the control except at the 10th week storage post-treatment where all the microbial isolates showed slight significant differences in reduced germination performance when compared with the control.

CONCLUSION

This study concludes that isolates of *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii* and *Bacillus thuringensis* do not reduce germination performance of cowpea seeds when used as microbial pesticides against cowpea storage pests. However, at longer storage post-treatment of cowpea seeds with these entomopathogenic isolates, the germination performance may be reduced.

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