



BIOACTIVITIES OF SOME ORGANIC AGENTS ON SEED BORNE MICROBES ASSOCIATED WITH BLACK VELVET TAMARIND (*DIALIUM GUINEENSE*)

OSAIGBOVO AU^{a*} • AKAEZE OO^a • OSAGIE JO^a • ADEKUNLE AT^a • LAW-OGBOMO KE^a • ISIKHUEMEN EM^b

^a Department of Crop Science, University of Benin, Benin City, Nigeria.

^b Department of Forest Resources and Wildlife Management, University of Benin, Nigeria.

*Correspondence author. E-mail: agbonsalo.osaigbovo@uniben.edu

ABSTRACT: This study was conducted to isolate and identify microbes (fungi and bacteria) associated with the seeds of *Dialium guineense* and to evaluate some organic control agents (neem, honey and neem+honey (ratio 1:1)) for bioactivities. The experiment was laid in a completely randomized design for evaluation of bioactivity against fungi and a 3X4 (three organic control agents X four levels of concentration) factorial in CRD for evaluation of organic control for bioactivity against bacteria, both experiments were replicated three times. Data were analyzed by analysis of variance and means were separated using Duncan multiple range test (DMRT) A total of six bacteria (*Klebsiella spp.*, *Bacillus spp.*, *Staphylococcus spp.*, *Pseudomonas spp.*, *Streptococcus spp.*, *Escherichia coli*) and six fungi (*Neurospora spp.*, *Fusarium spp.*, *Penicillium spp.*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceous*) were isolated from seeds of *Dialium guineense* collected from Awka market, Oje-Oba market, Uselu market and Ekpoma markets, in Anambra, Ondo and Edo states, Nigeria. Percentage fungal seed infection was high in all location with seeds from Ekpoma market having the highest infection of 77.08 while seeds from Oje-Oba market having the least in infection occurrence of 24.97. There were no significant differences for bacterial infection for seeds obtained from Awka market and Ekpoma markets (29.33), but seeds from Uselu market recorded the highest bacterial infection rates of 73.57. Generally, lower concentrations of neem and honey extracts showed more bioactivity (1.10 and 1.07 respectively) against bacterial isolates while Neem+honey recorded highest bioactivity at the highest concentration of 30% (1.30) Bioactivity of organic control agents was highest at the first day after inoculation but declined over time. Based on the findings of this study neem extracts at low concentrations of 01% is recommended for use as in the controlling seed borne fungi of *Dialium guineense*

Key words: Zone of inhibition, Isolates, Plant extract, Anti-microbial, Concentration

1. INTRODUCTION

Black Velvet Tamarind (BVT) (*Dialium guineense*) belongs to the family *Fabaceae* and subfamily *Caesalpinioideae*. It is an important non-timber multipurpose agro forest crop. BVT is widely distributed in the tropics (Orhue *et al.* 2007: Ikechi-Nwaogu and Nwaukwu 2012) and is ranked one of the most consumed wild fruits in Nigeria (Ogbe and Egharevba 1992). The roots, barks and leaves have medicinal values and are used as treatment of fever, stomach ache, mouth wash, gargle for sores, toothache and also in the easing of labour pain (Nwaoguala *et al.* 2007). Obaseki-Ebor *et al.* (1993) reported that extracts from the leaves of Black Velvet Tamarind serves as a potential anti-mutagenic remedy against tumors and other malignant cells.

Despite the potentials and high consumption of BVT, it still remains one of the lesser known tropical fruit as

it is given less priority in terms of research, production, improvement, storage and preservation (Ogbe and Egharevba 1992). The increasing large scale deforestation, poor seedling germination, slow seedling growth, long germination period of seed sown and over exploitation from its natural habitat, increases its chances of extinction (Osaigbovo and Law-Ogbomo 2012). Seeds, the cheapest inputs in crop production, are the major means of propagation of BVT. The association of certain microbes with seeds has often resulted in significant loss in seed quality, nutritional composition of seeds, crop yields, and seed viability. It may also result in reduced seedling vigor, seed discoloration and shriveling and long term development of plant disease (Koirala *et al.* 2005). According to Umoru and Benjamin, (2015) good quality seeds must among other things, be free of disease causing pathogens. Different methods such as the use of synthetic fungicides, heat treatment, biological control agents, plant leaf extract etc. have

been used in the past for their effectiveness to control seed borne pathogens (Valeria and Gianfranco 2013; Okigbo and Ogbonnaya 2009). The objectives of this study were to isolate and identify fungi and bacteria isolates associated with the seeds of *Dialium guineense* and to evaluate the efficacy of some organic control agents for bioactivity against isolated microbes.

2. MATERIALS AND METHODS

Dialium guineense fruits were obtained from from Awka market, Oje-Oba market, Uselu market and Ekpoma markets, in Anambra, Ondo and Edo states, Nigeria. Seeds were extracted from fruit, and air-dried for 72 hours. *Azadirachta indica* (leaf), pure honey, and *Azadirachta indica* leaf and honey extract mixed in the ratio 1:1 were evaluated for their antimicrobial potentials. Neem extract was prepared by grinding to powdered form neem leaves that have been harvested and air-dried for 14 days. Two hundred and fifty (250) grams of neem powder was then dissolved in seven hundred and fifty (750) milliliters (ml) of ethanol and was sieved through whatman No.1 filter paper. The filtrate obtained was then evaporated to dryness using a rotary evaporator. Stock solution (5% w/v) of the neem, honey and neem+honey (1:1) was prepared by dissolving 2.2g of each organic control agent in 20ml of sterile distilled water. Concentrations of 10, 20, and 30% were prepared from the stock solutions by dissolving 2, 4, and 6ml of each stock solution in 18, 16 and 14ml ml of sterile distilled water respectively. Microbial isolation for fungi and bacteria from seeds were done by direct plating method on potato dextrose agar (PDA) and nutrient agar (NA) respectively as described by Okhuoya *et al.* (2012). Purification of cultures was done by sub culturing. Fungal identification was done by observing cultural and morphological characteristics of pure culture using the key described by Barnett and Hunter (1998). Bacteria identification was done by Gram staining after which catalase, oxidase, coagulase, urease, citrate, indole tests were carried out as described by Christopher and Bruno (2003) where appropriate. Evaluation of organic agents for bioactivity against microbes was done by agar dilution and agar well diffusion methods for fungi and bacteria tests (Okhuoya *et al.* 2012). The experiment was laid in a completely randomized design (CRD) for evaluation of bioactivity against fungi and a 3X4 (three organic control agents X four levels of concentration) factorial in CRD for evaluation of extracts for bioactivity against bacteria isolates. Both experiments were replicated three times.

Data on percentage infection frequency was first transformed with Arcsine and then all data were subjected to analysis of variance (ANOVA) and significantly different means were separated using Duncan multiple range test (DMRT) at 5% level of probability.

3. RESULTS AND DISCUSSION

A total of six bacterial isolates namely *Klebsiella spp.*, *Bacillus spp.*, *Staphylococcus spp.*, *Pseudomonas spp.*, *Streptococcus aureus* and *Escherichia coli* were isolated from seeds of *Dialium guineense* from four locations in Nigeria. *Klebsiella spp.* and *Bacillus spp.* were isolated from seeds obtained from 'Awka' market. *Klebsiella spp.*, *Bacillus spp.*, *Staphylococcus aureus* and *Pseudomonas spp.*, were isolated from 'Oje-Oba' market. *Bacillus spp.* alone was isolated from 'Uselu' market, while *Streptococcus spp.*, *Escherichia coli*, *Klebsiella spp.* were isolated from 'Ekpoma' Market respectively. *Klebsiella spp.* and *Bacillus spp.* were recurrent in three out of the four markets sampled. Seeds sampled from 'Uselu' market had the least bacteria diversity with only *Bacillus spp.* isolated from them while seeds from 'Oje-Oba' market had the highest bacteria diversity with four different bacteria species isolated (Table 1).

Six fungal isolates - *Neurospora spp.*, *Fusarium spp.*, *Penicillium spp.*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus* (table 1) were isolated and identified from the seeds of *Dialium guineense* collected from four markets in Nigeria. *Neurospora spp.*, *Fusarium oxysporum*, and *Aspergillus spp.* were isolated from 'Awka'- market. *Penicillium spp.* and *Aspergillus flavus* were isolated from 'Oje-Oba' market, while., *A. ochraceus* and *Penicillium spp.* were isolated from seeds from 'Uselu' market. *A. niger*, and *A. flavus* were found to be associated with seeds from 'Ekpoma' market. The percentage microbial infection of *Dialium guineense* seeds from the four locations is presented in table 2. There was significant difference in the percentage fungal infection from all locations. The highest fungal infection rate was observed in seeds from 'Ekpoma' market (77.08) followed by 'Awka' market (75.82). These locations ('Ekpoma' market and 'Awka' market). However, showed the least percentage bacterial infection and were not significantly different from each other but were significantly lower than values obtained from Benin City and Akure (73.57 and 66.89).

Table 1: Fungi and bacteria isolates isolated from *Dialium guineense* seeds from four markets in Nigeria

Locations	Fungi Isolates	Bacteria isolates
'Awka' Market (Awka)	<i>Neurospora spp</i> <i>Fusarium spp</i> <i>Aspergillus niger</i>	<i>Klebsiella spp</i> <i>Bacillus spp</i>
'Oje-Oba' Market (Akure)	<i>Aspergillus ochraceus</i> <i>Pencillium sp</i>	<i>Bacillus spp</i>
Ekpoma Market (Ekpoma)	<i>Aspergillus niger</i> <i>Aspergillus flavus</i>	<i>Streptococcus spp</i> <i>Escherichia coli</i>

Plate 1a: Fungal isolation from seeds of *Dialium guineense* using direct plating (on potato dextrose agar) methodPlate 1b: Bacterial isolation from seeds of *Dialium guineense* using direct plating (on nutrient agar) method

Table 2: The percentage infection on the seeds by fungi and bacteria pathogens

Seed sources	Fungi (%)		Bacteria (%)	
	X	X ⁻¹	X	X ⁻¹
Awka market	94	75.82 ^b	24	29.33 ^c
Oje-Oba market	22	24.97 ^d	84.62	66.89 ^b
Uselu market	67.73	55.12 ^c	92	73.57 ^a
Ekpoma market	95	77.08 ^a	24	29.33 ^c
LSD (0.05)		0.019		0.019

X= Actual value, X⁻¹= Arcsine value, means followed by different letter(s) along the column are significantly different at 5% level of probability.

Table 3 shows the effect of concentration of organic control agents (Neem, Honey and Neem+Honey) on the growth (cm) of *Fusarium oxysporum* over a three day period. At day 1, all levels of concentration of neem leaf extracts significantly inhibited growth of *F. oxysporum* with mean growth of 0.80, 0.70, and 0.83 for 10, 20 and 30% concentration respectively compared with the control value of 1.50. The extract did not effectively inhibit growth of *F. oxysporum* at the second and third day after inoculation. Honey at 10% concentration inhibited growth of *F. oxysporum* (1.17) on the first day after inoculation, however, further increase in concentration and days after inoculation resulted in reduced bioactivity. Concentration and days after inoculation did not have any significant effect on the antimicrobial properties of Neem+Honey on *F. oxysporum*.

Neem leaf extract significantly reduced the growth of *Neurospora spp.* at all levels of concentration. However, increased bioactivity was observed per increase in concentration applied. The highest inhibition level of 1.76 against the control value of 5.03 was observed at the highest concentration of 30%. Increase in days resulted in reduced bioactivity. There was no significant effect of concentration of honey over the three-day period on the inhibition of *Neurospora spp.* All concentrations of Neem+honey significantly inhibited growth of *Neurospora spp.*, but the highest inhibition of 3.20 against the control value of 5.03 was observed at the 20% concentration on the first day after inoculation but bioactivity declined on the second and third day after inoculation.

The growth of *A.niger* was inhibited by neem leaf extract throughout the three day period. Bioactivity was however high at the highest concentration of 30%. Low bioactivity was recorded for honey except for 20% treatment (4.53) on the third day which was significantly lower than the control (5.73). Neem+honey showed more antimicrobial properties at lower concentrations of 20% (1.37), 10% (2.70) 10% (4.27) for day 1, 2, and 3 respectively.

Aspergillus flavus was significantly inhibited by neem extract at the highest concentration of 30% (0.77) on the day 1 and 3 after inoculation; however, there was no significant inhibition in the growth on the second and third day. Honey extract showed bioactivity against *A.flavus* at 20% (0.80 and 2.03) on the first and third day respectively. The bioactivity of neem+honey at low concentration of 10% increase over time with growth values of 1.37 and 1.80 compared with control values of 1.93 and 2.93 for day 2 and 3 respectively.

A.indica leaf extract evaluated against *A. ochraceous* showed significant level of bioactivity at 20% concentration at day 1 and 3 after inoculation. No significant inhibition was observed on the second day across all concentrations. Honey did not significantly inhibit growth of *A. ochraceous* across all concentrations over the three day period. Neem+honey treatment was most effective at lower concentration of 10% (2.7) on the third day when compared to the control value of 7.50.

The antimicrobial properties of *A.indica* leaf extract on *Penicillium sp.* increased by concentration and time, with the highest inhibition recorded for the highest concentration of 30% on the second and third day. The bioactivity of honey increased at lower concentration over time with low concentration of 10% having the highest growth inhibition of 0.47 and 1.00 against control values of 1.17 and 2.33 for day 2 and 3 respectively. Antimicrobial activity of neem+honey was highest at 30% concentration on day 2 and 3 with growth values of 0.60 and 1.80 compared with control values.

Table 4 indicated that there were no significant differences in the bioactivities of neem, honey and neem+honey against *Bacillus spp.*, *Klebsiella spp.*, and *Pseudomonas spp.* *A. indica* leaf extract showed highest bioactivity against *Staphylococcus spp.*, *E.coli*, and *Streptococcus spp.*, but was not significantly different from neem+honey. Extracts at low concentration of 10% showed significant antimicrobial activities against *Bacillus spp.*, *Staphylococcus sp.*, *Streptococcus spp.*, *Pseudomonas spp.*, and *E.coli*. Levels of concentration did not influence the level of bioactivity of extracts against *Klebsiella spp.*

Table 5 shows the interaction effects between organic control (extracts) and the level of concentration on growth inhibition of bacterial isolates. Concentrations of neem and honey had no significant effect on the bioactivity against *Bacillus spp.* while 10% concentration of neem+honey extracts significantly inhibited *Bacillus spp.* Although, concentration did not influence antimicrobial activities of neem and neem+honey extracts, honey at 10% concentration (0.37) significantly inhibited growth of *Pseudomonas spp.* compared to 20% (1.57) and 30% (1.47) respectively. Generally, lower concentrations of neem and honey showed more bioactivity (1.10 and 1.07 respectively) against bacterial isolates while Neem+honey recorded highest bioactivity at the highest concentration of 30% (1.30).

Table 3: shows the effect of the organic control agents on the growth of fungi pathogens

Treatment	<i>Fusarium sp</i> Growth			<i>Nerospora sp</i> Growth			<i>Aspergillus niger</i> Growth		
	Day1	Day2	Day3	Day1	Day2	Day3	Day1	Day2	Day3
Control	1.50 ^c	1.20 ^a	2.10 ^{ab}	5.03 ^e	7.33 ^a	7.33 ^a	1.63 ^c	3.27 ^{bc}	5.73 ^d
NEEM									
10%	0.80 ^a	1.80 ^b	2.37 ^{abc}	3.00 ^{bc}	8.23 ^{ab}	8.23 ^{ab}	1.47 ^{ab}	2.90 ^{abc}	5.30 ^{cd}
20%	0.70 ^a	1.77 ^b	2.70 ^c	2.23 ^{ab}	9.00 ^b	9.00 ^b	1.43 ^{ab}	2.80 ^{abc}	4.97 ^{bc}
30%	0.83 ^a	1.83 ^b	2.45 ^{bc}	1.767 ^a	8.07 ^{ab}	8.07 ^{ab}	1.33 ^a	2.73 ^{ab}	4.73 ^{abc}
HONEY									
10%	1.17 ^b	1.77 ^b	1.83 ^a	4.87 ^e	8.47 ^{ab}	8.47 ^{ab}	1.70 ^c	3.03 ^{abc}	5.10 ^{bcd}
20%	1.67 ^c	3.03 ^{cde}	4.20 ^e	4.70 ^{de}	8.50 ^{ab}	8.50 ^{ab}	1.57 ^{bc}	3.30 ^c	4.53 ^{ab}
30%	1.63 ^c	3.13 ^{de}	4.37 ^e	4.90 ^e	8.50 ^{ab}	8.50 ^{ab}	1.67 ^c	3.27 ^{bc}	5.10 ^{bcd}
HONEY+NEEM									
10%	1.60 ^c	3.23 ^e	3.53 ^d	3.60 ^c	8.57 ^{ab}	8.57 ^{ab}	1.47 ^{ab}	2.70 ^a	4.27 ^a
20%	1.80 ^c	2.73 ^{cd}	2.73 ^c	3.20 ^c	8.93 ^b	8.93 ^b	1.37 ^a	2.77 ^{abc}	4.80 ^{abc}
30%	1.50 ^{bc}	2.70 ^c	3.87 ^{de}	3.80 ^{cd}	8.50 ^{ab}	8.50 ^{ab}	1.43 ^{ab}	3.00 ^{abc}	5.17 ^{bcd}
SIG	**	**	**	**	*	*	**	*	*
LSD	0.325	0.386	0.515	0.918	1.226	1.226	0.132	0.49	0.626

Means with the different letters along the columns are significantly different at 5% level of probability

Table 3 Cont'd

Treatment	<i>Aspergillus flavus</i> Growth			<i>Aspergillus ochraceus</i> Growth			<i>Aspergillus ochraceus</i> Growth		
	Day1	Day2	Day3	Day1	Day2	Day3	Day1	Day2	Day3
Control	1.00 ^{bde}	1.93 ^c	2.93 ^e	1.10 ^{cd}	1.80 ^a	7.50 ^d	0.40 ^a	1.17 ^{cd}	2.33 ^{fg}
NEEM									
10%	0.87 ^{abcd}	1.83 ^{bc}	2.40 ^{bc}	0.93 ^{ab}	2.17 ^a	3.60 ^{bc}	0.43 ^a	0.97 ^{bc}	1.97 ^{bcd}
20%	0.87 ^{abcd}	1.60 ^{abc}	2.47 ^{bcd}	0.83 ^a	1.97 ^a	3.17 ^{ab}	0.47 ^a	1.07 ^{bcd}	1.83 ^{bcd}
30%	0.77 ^a	1.70 ^{abc}	2.37 ^{bc}	0.83 ^a	1.93 ^a	3.47 ^{bc}	0.53 ^a	0.90 ^b	1.70 ^b
HONEY									
10%	1.03 ^e	1.93 ^c	2.83 ^{de}	1.20 ^d	2.23 ^a	3.73 ^c	0.43 ^a	0.47 ^a	1.00 ^a
20%	0.80 ^a	1.80 ^{bc}	2.03 ^{ab}	1.13 ^{cd}	1.80 ^a	3.70 ^c	0.43 ^a	1.20 ^d	2.10 ^{def}
30%	1.13 ^e	2.33 ^d	2.57 ^{cde}	1.20 ^d	2.17 ^a	3.77 ^c	0.40 ^a	1.27 ^d	2.53 ^g
HONEY+NEEM									
10%	0.83 ^{ab}	1.37 ^a	1.80 ^a	1.03 ^{bc}	2.10 ^a	2.77 ^a	0.43 ^a	1.13 ^{cd}	2.03 ^{cd}
20%	0.83 ^{abc}	1.53 ^{ab}	2.20 ^{abc}	1.10 ^{cd}	2.37 ^a	3.67 ^{bc}	0.43 ^a	0.60 ^a	2.13 ^{ef}
30%	0.83 ^{abc}	1.73 ^{bc}	2.23 ^{bc}	0.97 ^b	2.33 ^a	3.63 ^{bc}	0.50 ^a	0.97 ^{bc}	1.80 ^b
SIG	**	**	**	**	ns	**	ns	**	**
LSD	0.149	0.325	0.39	0.12	0.64	0.464	0.146	0.2	0.27

Table 4: Growth inhibition of bacterial isolates from *Dialium guineense* seeds using organic agents

Treatment(E)	<i>Bacillus spp</i>	<i>Staphylococcus spp</i>	<i>Klebsiella spp</i>	<i>Streptococcus spp</i>	<i>Pseudomonas spp</i>	<i>E.coli</i>
Neem	1.30 ^a	1.28 ^b	1.28 ^a	1.06 ^c	1.09 ^a	1.29 ^b
Honey	1.33 ^a	1.68 ^a	1.46 ^a	1.69 ^a	1.13 ^a	1.46 ^b
Neem+ Honey	1.29 ^a	1.36 ^b	1.32 ^a	1.36 ^b	1.21 ^a	1.79 ^a
Sig.	Ns	**	Ns	**	ns	**
LSD (P<0.05)	--	0.229	--	0.116	--	0.189
Conc. (C)						
10%	1.16 ^b	1.27 ^b	1.48 ^a	1.30 ^b	0.82 ^b	1.36 ^b
20%	1.26 ^b	1.50 ^a	1.29 ^a	1.30 ^b	1.32 ^a	1.84 ^a
30%	1.51 ^a	1.54 ^a	1.29 ^a	1.50 ^a	1.29 ^a	1.33 ^b
SIG	**	*	Ns	**	**	**
LSD	0.122	0.229	--	0.116	0.249	0.189
E x C	**	Ns	Ns	ns	**	**

Means with the different letters along the columns are significantly different at 5% level of probability

Table 5: Inhibition zones (cm) from organic agents and their interactions on bacteria isolated from *Dialium guineense* seeds

Control agents	Levels of conc.	<i>Bacillus sp</i>	<i>Pseudomonas sp.</i>	<i>Escherichia sp</i>
Neem	10	1.00 ^d	0.97 ^c	1.10 ^c
	20%	1.20 ^{cd}	1.13 ^{bc}	1.77 ^{ab}
	30%	1.70 ^d	1.17 ^{abc}	1.00 ^c
Honey	10%	1.30 ^{bc}	0.37 ^d	1.07 ^c
	20%	1.33 ^{bc}	1.57 ^a	1.60 ^b
	30%	1.37 ^{bc}	1.47 ^{ab}	1.70 ^b
Neem + Honey	10%	1.17 ^{cd}	1.13 ^{bc}	1.90 ^{ab}
	20%	1.23 ^c	1.27 ^{abc}	2.17 ^a
	30%	1.47 ^b	1.23 ^{abc}	1.30 ^c
SE		0.011	0.145	0.111

Means with different letters along the columns are significantly different at 5% level of probability



Plate 2:

- 1: agar well containing sterile distilled water (control) with no clear inhibition zone
- 2: agar well with honey, with observable zone of inhibition
- 3: agar well containing neem+honey extract with clear zone of inhibition
- 4: agar well containing neem extract with clear zone of inhibition.

Fungi and bacteria isolates have been associated with seeds of numerous plants in Edo state and other locations (Adekunle and Ndego 2007; Amadi *et al.* 2009; Miller, 2014). In this study, fungi isolated from seeds of *Dialium guineense* included *Neurospora spp.*, *Fusarium spp.*, *Pencillium spp.*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus*. This agrees with the findings of Adekunle (2004) who isolated *Aspergillus species*, *Penicilium sp.*, *Fusarium solani*, *Phoma sp.*, and *Geotrichum spp.*, from seeds of citrus species. Bacteria isolated from seeds of *Dialium guineense* included *Klebsiella spp.*, *Bacillus spp.*, *Staphylococcus spp.*, *Pseudomonas spp.*, *Streptococcus aureus*, *Escherichia coli*.

The use of plant extracts and other organic agents for their bioactivity against microbes has been well documented. An *in-vitro* and *in-vivo* study on antifungal properties of plant extracts against *Dreschlera heveae* showed that plant extracts showed

significant bioactivity against *D. heveae* (Ogbebor and Adekunle 2008; Ogbebor *et al.* 2005). In this study, neem extract showed promising antimicrobial properties against fungal isolates. This confirms the findings of Adeyeye and Olufolaji (2002), Ogbebor and Adekunle, (2005) and Ojo and Olufolaji (2005) on the efficacy of the bioassay of some plants extracts on selected fungal pathogens. Honey had more bioactivity against bacterial isolates than fungal isolates. This may be attributed to its enzymatic production of hydrogen peroxide, low pH, and the osmotic effect of its high sugar content and low moisture content (Mandal *et al.*, 2010; Mandal *et al.* 2011; Haniyeh *et al.*, 2010; Lusby *et al.* 2005; Kwakman *et al.* 2010).

The organic control agents evaluated recorded the highest bioactivity at the first day after inoculation and declined over time. This trend was also observed by Adekunle and Enobakhare (2003) who evaluated the fungicidal properties of *Acalypha wilkesiana* on *Fusarium spp.* the extracts was observed to lose bioactivity over time. Raji *et al.* (2005) suggested that this may be because some of the compounds responsible for the antifungal properties in plant materials are water soluble or volatile under sub-optimal conditions.

4. CONCLUSION

1. Fungi and bacterial isolate were found to be associated with seeds of *Dialium guineense*.
2. Organic agents (Neem, Honey and Neem+Honey (1:1)) evaluated showed varied level of bioactivity against isolated microbes
3. The level of bioactivity of control agents used were influenced by the level of concentration.
4. Organic control agents were more effective on the first day after application, but declined over time.

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