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Efficacy of four plant emulsions for managing long tail mealybugs (*Pseudococcus longispinus*) infesting banana plantations in Cameroon

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Abstract
From November 2013 to May 2014, the insecticidal properties of detergent-oil (mixture of liquid washing detergent and vegetable oil) emulsions of four plants (*Lantana camara, Allium sativum, Coffea arabica, and Jatropha curcas*) were tested on the long tailed mealy bugs infesting banana plants in Cameroon. The treatment doses used in the laboratory were 5g, 10g, 15g of the plant powders per 100mL of 15% detergent-oil mix, 2mL of chlopyrifos-ethyl in 500mL (positive control), and untreated tap water (negative control). In the field, the treatments were; an untreated control (nothing applied), 15g of *Lantana* per 100ml of 15% of detergent solution, 10g of Garlic per 100ml of 15% of detergent solution, 15g of coffee husk per 100ml of 15% of detergent solution, 15g of *Jatropha* per 100ml of 15% detergent solution, and 2ml of Pyriforce® (chlopyrifos-ethyl) per 500ml of water. In the laboratory, the results for the plant emulsions showed that *L. camara* (range of 64.5-94.5%) and *A. sativa* (range of 88.9 – 100%) emulsions had the highest mortality. As for repellency, the highest was from *J. curcas* treatment (72 – 92%), In the field, all the doses of the plant emulsions used significantly reduced the mealy bug infestation as compared to the positive control (water). The different plant emulsions showed high potential to kill and repel mealy bugs. Combining *J. curcas* with either *L. camara* or *A. sativum* could be evaluated for the management of mealy bugs in the fields.

Keywords: Mealy bug, banana, botanical, emulsion, pesticide

1. Introduction
Banana is a major source of revenue for Cameroon with production of about 249,610 tons exported in 2016 and 279,493 tons in 2015, putting the country in second place behind Cote D'Ivoire in Africa [1]. The production of banana in Cameroon has been increasing steadily from 2001 (631,766 tons) to 2016 (1,187,547 tons), occupying the 15th position in the world with 1.5% share [2]. Generally, bananas and plantains play vital roles in maintaining food/nutrition security in most developing countries especially in Sub-Saharan Africa [3]. In addition to its export value, banana plantations and smallholder plantain farms serve as important source of employment [3, 4]. In Cameroon, banana plantations are mostly in the Littoral and South West Regions and these agro-industrial farms are usually faced with major biotic constraints to production such as corm weevils, burrowing nematodes, black sigatoka, and recently, mealy bugs, aphids, white flies and snails [5]. Mealybug infestation became serious in 2007 as this sap suckers and honey-dew producing insects emerged as an important economic pest in all the banana plantations in Cameroon [3]. They are mainly found within the pseudostems although juveniles occasionally move into the bagged bunches, where they find favourable microclimate for rapid reproduction and honey dew production [3]. Fermentation of the honey dew leads to the production of sooty mold on the fruits which can be so intense that it cannot be removed even by the high pressure water and washing in the pack houses. This ultimately results in rejection of such fruits for export, leading to losses that probably vary from 0.3% to 30% depending on the farm management techniques, climate and seasons (i.e. high infestation during the dry season) [4].

Despite the adoption of cultural techniques such as the removal of old pseudostem sheaths and the use of chlopyrifos-treated bags to protect banana fruits, mealybug infestation has persisted and has become a critical fruit quality issue in banana plantations.

Mealybug infestation in banana plantations in Cameroon is controlled using synthetic pesticides such as Dursban® with active ingredient Chlopyrifos. However, since these insects are mostly concentrated on bunches, direct application of Dursban® on banana fruits can result in large amounts of pesticide residues which are not safe for human consumption [4]. Considering the risks of synthetic pesticides on human health, beneficial non-target organisms and possibility for pest evolution and resistance to pesticides [5], there is increasing need for sustainable alternative management strategies including plant-derived extracts that are environment-friendly and safe for consumers [6]. Detergents and soaps have demonstrated fatal effects on insect pests with less negative effect on the environment [7]. Furthermore, many plant-derived extracts (botanicals) have been used to successfully manage field pests without negative consequences [8-10]. In Africa, botanicals (i.e. *Lantana camara, Allium sativum, Jatropha curcas, Ocimum gratissimum, neem,* etc.) reportedly control pest infestations and disease incidences [11]. Meanwhile, the combination of detergents and vegetable oil has been used as emulsifier concentrate (adjuvants) for botanicals to enhance their performance on insect pests [12].

It was hypothesized that garlic and *Lantana* emulsions will enhance mealybug mortality and/or repulsion in the laboratory and effectively reduce mealybug population in the field compared to synthetic pesticide. Considering the effectiveness of emulsions as adjuvants for botanicals and the quest for effective local botanicals, this study was conceived to determine the most effective application dose of four plants (*Jatropha* leaves, *Lantana* leaves, coffee husks and garlic bulbs) emulsions and evaluate their efficacy as botanical pesticides for managing mealybug infestation on banana plants.

2. Materials and Methods

2.1 Study site

The present experiment was conducted at the Research Station of the Institute of Agricultural Research for Development (IRAD) Ekona where environmental conditions (i.e. warm temperatures and abundant rainfall) favour the growth of plantains and bananas. Ekona is located in the South West Region of Cameroon, precisely in Muyuka Sub Division of Fako Division. It is situated at coordinates 4°16'44" N and 9°17'50" E in DMS (Degrees Minutes Seconds). This region has two main seasons: a wet season with rains from March to October and dry season from November to February. The mean annual rainfall varies between 2085 mm near Ekona on the leeward side, to 9086 mm at Debunscha on the windward side of Mount Cameroon [13]. Temperatures are higher than in the other areas of the southern part of the country with mean annual temperature that varies between 26.7 to 27.6 °C, with the minimum temperatures in August and the maximum temperatures in February and March [14]. It has been shown that these soils have poor water holding capacity [15] andthe soil temperature at 10 cm depth varies from 25 °C at 200 m above sea level through 20 °C at 1100 m altitude to 15 °C at 2200 m altitude [16]. The site has mean annual relative humidity of 76-90%.

2.2 Mealybug collection and culture

Mature mealybugs that were not attached to egg sacs were collected by cutting portions of infested pseudostems or carefully removing them (using thin plastic slender stripes from a new toothbrush) from bunch stalks from banana plants at the Cameroon Development Corporation - CDC plantations and cultured in the laboratory of Entomology at IRAD Ekona. This was carried out by placing 5 cm pseudostems pieces into 5 L transparent bucket and pseudostems containing mealybugs were added in the buckets.

2.3 Collection of Botanicals and Synthetic Pesticides

Plant biomass (botanicals) included leaves of *Jatropha curcas* and *Lantana camara*, husks of *Coffea arabica* and bulbs of *Allium sativum.* Leaves of *Jatropha* and *Lantana* were harvested by cutting with a cutlass from hedges at IRAD Ekona, coffee husks were collected from a coffee milling factory at Ekona, and garlic bulbs were obtained from commercial suppliers at Ekona market. The organophosphate synthetic pesticide - Pyriforce® (Made in France; comprising 600 g/L Chlorpyriphos-Ethyl as active ingredient) was obtained from an agrochemical shop.

2.4 Laboratory experiments

2.4.1 Preparation of plant extracts

The plant biomasses were thoroughly washed to remove impurities like debris and air-dried under shade for seven days. The dry plant biomasses were ground to very fine powder using a locally fabricated electrically controlled corn milling machine (made in Cameroon). The machine was washed and dried after grinding each plant biomass and sterilized with chlorine detergent (Eaux de Javel; Clorox®, USA) before being used for the next plant biomass. The emulsifier concentrate was prepared by mixing 60ml liquid washing detergent (Liquide Vaisselle®, Made in France) and 90ml soy bean oil (Oilio®, Made in Cameroon) in 1 L tap water. Three doses of each plant extract were prepared by adding 5g, 10g and 15g of the plant powders in 100ml emulsifier concentrate to produce the respective plant emulsions.

2.4.2 Assessment of botanicals for mortality and oviposition of mealybugs

There were five treatments (T) for each plant emulsion with three replicates each:

T1 = Negative control (water)
T2 = (5g plant emulsion)
T3 = (10g plant emulsion)
T4 = (15g plant emulsion)
T5 = the positive control (2ml of chlorpyriphos-ethyl per 500ml of water).

The plant extracts were put in 1 Litre plastic bottles and allowed to stand overnight for thorough mixing before use. Fresh pseudostems were collected from banana plants using a knife and divided into smaller pieces of 5cm each. They were immersed in the extracts for 10 minutes, air-dried for 10 minutes and placed into labelled transparent plastic boxes of 13cm x 13cm x 7cm dimensions with perforated lids. Using a thin plastic slender stripe from a new toothbrush, ten adult female mealybugs were gently placed on the pseudostems. Only females were used for this research since males naturally are very scarce. This procedure was performed for all the different botanicals and the setup was monitored daily for mortality over 7 days at mean temperature of 25.3°C, mean relative humidity of 82.1% and photo period of 13 light hours and 11 dark hours. Data on the number of dead mealybugs and the number of egg sacs produced were recorded daily. Each experiment with its replicates was repeated three times.

2.4.3 Assessment of botanicals for repellency
The treatments, replicates and procedure were the same as described above for mortality and oviposition. However, instead of putting the mealybugs on the treated pseudostems, they were placed 3 cm away from the treated pseudostems. The number of mealybugs that stayed away from the treated pseudostems was recorded daily for 7 days andthe entire experiment was also repeated three times.

2.5 Field experiment
The present experiment was carried out in a newly planted banana farm which was about 500 m away from other plantain and banana fields with well-drained soil and level topography. The experiment was carried out for 5 months (December 2013 to May 2014 – usually involving the dry season with higher infestation and part of the rainy season). 180 holes were dug at a diameter of 20cm and 15cm deep. Banana suckers were gotten from the banana plantation of CDC, pared and planted into the holes. Before planting, the old leaf sheaths were removed and the suckers washed thoroughly with tap water to remove any mealy bugs present. NPK (20-20-10) fertilizer was applied once at the first month of planting at 20g per plant by ringing. Manual weeding of the farm was done by slashing using a cutlass.
The field experimental setup was a randomized complete block design with six treatments comprising the most effective doses from the laboratory experiments and replicated three times each. Each replicate consisted of 10 plants, giving a total of 30 plants per treatment. Each plant was tagged for easy identification using boldly labeled papers placed in transparent plastics to avoid destruction by rain. The plant emulsion for field application was prepared by adding the most effective dose of the respective plant powder in 100ml emulsifier concentrate to produce the respective plant emulsions in the treatments (T) below:
T1 = Untreated control (nothing applied)
T2 = 15g *Lantana emulsion*
T3 = 10g *Garlic emulsion*
T4 = 15g *Coffee emulsion*
T5 = 15g of *Jatropha emulsion*
T6 = 2ml Pyriforce® (chlopyrifos-ethyl) per 500ml of water

One week before the banana plants were infested with mealybugs, their pseudostems were examined for occurrence of mealybugs and cleaned thoroughly by removing all observed mealybugs. The experimental plants were then infested by placing small pieces of pseudostems containing 50 long-tailed mealybugs between the dry and fresh leaf sheaths of the pseudostems. One month later, the plants were sprayed with the respective treatments using a hand sprayer. 250ml of each treatment solution was applied on the pseudostems (0.3 metres from the soil) of each plant and this was performed once a month. Each week, the pseudostems (30 cm above the soil) of the plants were examined using a hand lens and the number of mealybugs on the pseudostems were counted for 20 weeks. A tape was used to measure the height and circumference of the sample area for each plant. The density was obtained by counting the number of mealybugs in a measured 10cm x 10cm area within the sampled area and multiplied by the total sampled area.
Density in Sampled area = Density in 10cm x 10cm area x Sampled area
Sampled area = $2\pi r^2 + h\,(2\pi r)$ (mathwarehouse.com) where $\pi = 22/7$, r = radius, h = height.
$2\pi r^2 + h\,(2\pi r)$ is the formula of a cylinder (pseudostem considered as a cylinder).

2.6 Statistical analysis
Statdisk software version 9.1 [17] was used for Statistical analysis. Means, variance and standard deviations within the treatments were calculated using descriptive statistics. The different means were compared by One-way Analysis of Variance (ANOVA) while Posthoc (Tukey's pair-wise) comparison test at P =0.05 was used to determine the significant differences among the calculated means of the various treatments.

3. Results
3.1 Effects of botanicals on mealybug mortality and repulsion in the laboratory
3.1.1 *Lantana camara*
L. camara was very effective in killing mealy bugs especially at higher concentration, which resulted in higher mortality with up to 94% for both 10g and 15g (Fig. 1). There was a significant difference (P = 0.05) between the negative control (water) and the various doses of the plant extracts but there was no significant difference (P =0.05) when the different plant doses were compared with the positive control (2ml of Pyriforce). Of all the treatments of *L. camara*, 5g had relatively lower mean percentage mortality for mealy bugs.
Lantana camara repelled up to 49% of the population in the plant emulsions, which was significantly different from the negative control (20%). Furthermore, the ability to repel mealy bugs increased as the dose increased (Fig. 5) and the higher doses of *L. camara* (10g and 15g) were significantly different from the positive control (2mL of Pyriforce)(P = 0.05)

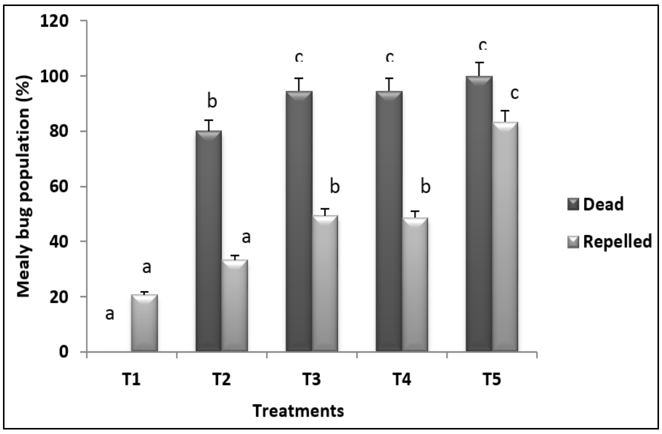

Fig 1: Percentage (Mean ± SD) dead and repelled mealybugs placed on pseudostems treated with different doses of *Lantana camara*; T1 = Negative control – water, T2 = 5g *Lantana* emulsion, T3 = 10g *Lantana* emulsion, T4 = 15g *Lantana* emulsion, T5 = Positive control – pyriforce; bars with the same letters are not significantly different according to Tukey's HSD P = 0.05.

3.1.2 Garlic (*Allium sativum*)
Garlic treatments demonstrated high mortality rates for mealy bugs with significant (P =0.05) difference between untreated control (water) and the different garlic treatments. Mortality increased as the concentration increased with the lowest dose (5g) having 88.9% and the highest (15g) having 100% mortality after 7 days (Fig. 2). However, there was no significant (P = 0.05) difference in mortality between the different doses of garlic emulsion and the synthetic pesticide. The repulsion varied for the garlic treatments (from 57.3 – 77.3%). Even 5g of garlic was able to cause a mean percentage repulsion of 57.3% on mealy bugs 7 days after treatment while 10g recorded a repellent effect of 77.3% (Fig. 2).

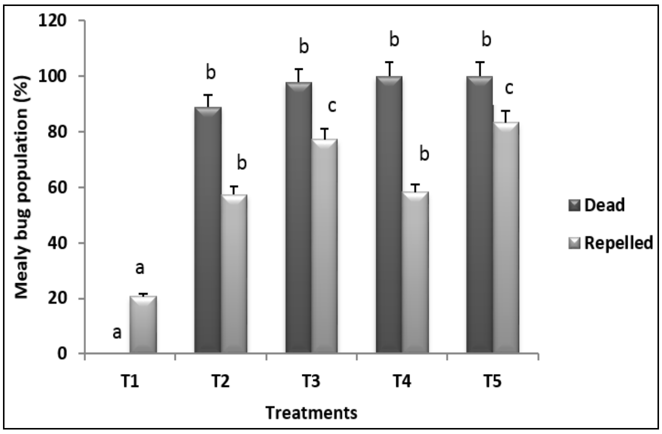

Fig 2: Percentage (Mean ± SD) dead and repelled mealybugs placed on pseudostems treated with different doses of garlic; T1 = Negative control – water, T2 = 5g garlic emulsion, T3 = 10g garlic emulsion, T4 = 15g garlic emulsion, T5 = Positive control – pyriforce; bars with the same letters are not significantly different according to Tukey's HSD *P* = 0.05.

3.1.3 Coffee husks (*Coffea arabica*)

Coffee husks had very low death rates with no significant difference (*P* = 0.05) between the various concentrations (Fig. 3) and the negative control (water). The highest concentration had only 5.7% mortality. Although coffee husks did not exhibit high mortality on the banana mealybugs, it had very high repellent effects, which is comparable to the effects of the synthetic pesticide. The lowest concentration of 5g coffee husks had 76% repellency after 7 days (Fig. 3). Mealybug repellency increased to 79.3% as the dose increased to 10g, but further increase in concentration did not influence repulsion. There was no significant (*P* = 0.05) difference between the positive control and the various coffee husks treatments for repulsion.

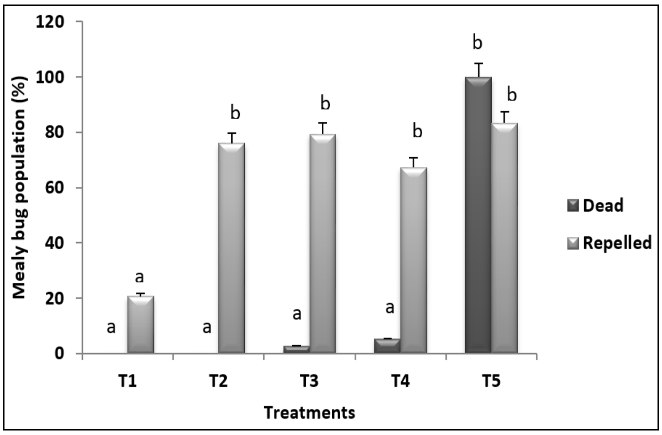

Fig 3: Percentage (Mean ± SD) dead and repelled mealybugs placed on pseudostems treated with different doses of coffee husks; T1 = Negative control – water, T2 = 5g plant emulsion, T3 = 10g plant emulsion, T4 = 15g plant emulsion, T5 = Positive control – chlopyrifos-ethyl; bars with the same letters are not significantly different according to Tukey's HSD *P* = 0.05.

3.1.4 *Jatropha curcas*

Mealybug mortality ranged from 0.7%-13.3% and all the three concentrations of *Jatropha* emulsion had similar mortality rates on mealybugs. There was no significant difference with the negative control but a significant difference occurred with synthetic pesticide as positive control (*P* = 0.05). While the synthetic pesticide had 40.7% mortality after the first day, *Jatropha* emulsion had less than 20% mortality. *Jatropha* emulsions however showed good repellent effects with the least dose (5g) having 72% and the highest dose had 92% after 7days (Fig. 4). There was no significant difference (*P* = 0.05) between 10g or 15g *Jatropha* emulsion and synthetic pesticide (Pyriforce®) positive control in repelling mealybugs, but there was a significant difference when compared with water as the negative control.

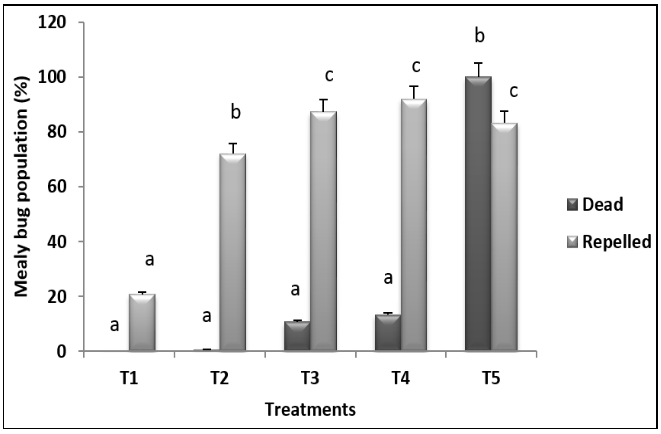

Fig 4: Percentage (Mean ± SD) of dead and repelled mealybugs placed on pseudostems treated with different doses of *J. curcas*; T1 = Negative control – water, T2 = 5g *Jatropha* emulsion, T3 = 10g *Jatropha* emulsion, T4 = 15g *Jatropha* emulsion, T5 = Positive control – chlopyriphos-ethyl; bars with the same letters are not significantly different according to Tukey's HSD *P*<0.05.

3.2 Effects of botanicals on mealybugs in the field

The mean mealybugs density ranged from 204.5 to 4488.6 across treatments, and there was a significant (*P* =0.01) difference between the various plant emulsions (botanicals) and the negative control (water) although these were not significantly different from the synthetic pesticide (P = 0.05). Population density for the various botanicals did not vary significantly from each other. However, coffee emulsion demonstrated the least effectiveness with high population density followed by *Jatropha* emulsion as compared to *Lantana* and garlic emulsions that had very low mealybug population in field.

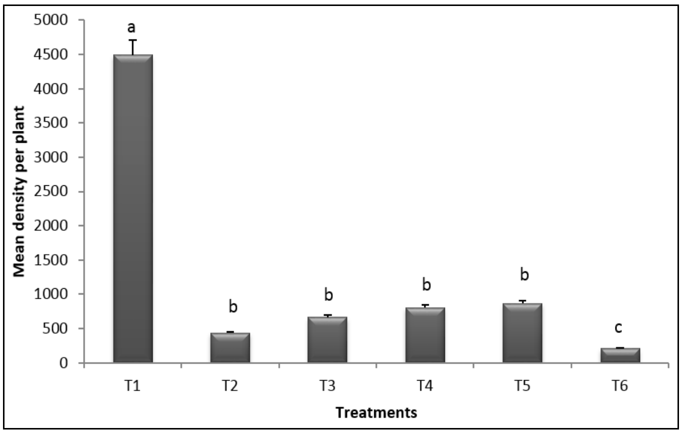

Fig 5: Number of mealybugs (Mean ± SD) on treated pseudostems 20 weeks after application of treatments in the field; T1 = untreated control, T2 = *Lantana* emulsion, T3 = Garlic emulsion, T4 = Coffee emulsion, T5 = *Jatropha* emulsion, T6 = Pyriforce; bars with the same letters are not significantly different according to Tukey's HSD *P* = 0.05.

4. Discussion

The results obtained in this study are in conformity with those of other studies which show that the botanicals used in the study can reduce the population of different types of pest [18-

[20]. Although all the plant emulsions used in this study showed varied mortality, *Lantana* and Garlic had greater mortality effects when the insects were placed directly on the treated-pseudostems. This indicates that botanical pesticides are mostly contact pesticides. This can be attributed to the fact that when these insects come in contact with plant extracts, they are poisoned, weakened and die.

The high mortality response of Lantana emulsion can be as a result of triterpene acids, lantadene A (rehmannic acid) and lantadene B which are secondary metabolites present in it [21, 22] also reported that aqueous extract of *L. camara* reduced the infestation of tea leaves against the tea mosquito bug - *Helopeltis theivora* [23]. Reported that 82.7% to 90.0% of *Sitophilus zeamais* mortality was caused by *L. camara* alone [24]. Studied the insecticidal action of aerial parts of *L. camara* against *Callosobruchus chinensis* (Coleoptera: Bruchidae) and found 10-43% mortality. The present researched shows clearly that *L. camara* has high mortality on mealybugs and was as effective as the synthetic insecticide (chlopyrifos-ethyl). This is in conformity with the work of [25] who increased the dose to 300mg of methanol extract of *L. camara* in 100ml of water to control *Aedes aegypti* and *Culex quinquefasciatus* [22]. Also confirms the findings of this research which showed *L. camara* as an insect repellant. They reported that *L. camara* does not only kill insects but also repels them and prevents them from feeding as well [25]. Concluded that application of *L. camara* strongly repelled mosquitoes.

The present research showed that garlic had a highest mortality rate and moderate repellency status. This may be attributed to the presence of allicin, a bioactive compound that is known to kill not only insects but also pathogens like fungi and bacteria [26, 28] did a similar study on the insecticidal activity of garlic juice on two dipteran pests and reported that garlic juice concentrate caused significant mortality in eggs and adults of the two insect species [27]. Found out that allicin does not kill by contact poisoning in the stomach but by inhibition of enzymes such as acetylcholinesterase [28]. Also makes it clear that certain concentrations of botanicals are equivalent to synthetic insecticides like organophosphates. For example, in the case of the *D. radicum* adults, a solution of only 0.8% of garlic was sufficient to give a mortality rate statistically indistinguishable from that of the organophosphate and this conforms with the results of this study where the dose of 15g emulsion had mortality rate of 3% different from the organophosphate insecticide (Pyriforce®) used as a positive control. Therefore, garlic juice or its emulsion may represent a direct replacement for more ecologically harmful conventional insecticides. The benefits of the commercial use of such a product could be significant as garlic would have little impact on the agricultural environment [29]. Confirms this result by describing the use of garlic as a good insect repellent and encouraged its use both for outdoor and indoor purposes. Since garlic acts by inhibition of enzyme [27], it can easily distort the egg formation process in insects thereby preventing oviposition. Overall, this result is consistent with the demonstrated performance of garlic extract as biopesticide against insect pests such as maize weevils and *Fulviafulva* of tomato [30, 31].

As for coffee husk, like the other botanicals used in this study showed a significant difference between its various concentrations and the negative control but did not have high mortality rates as the other botanicals (Fig. 3) [32]. Had similar results after carrying out in vitro studies on some natural beverages as botanical pesticides [18]. Used 10% coffee ground from café restaurants to control mealybugs, scales and aphids on cycads. The fact that coffee did not kill up to 20% of the mealy bugs within 7 could be that it effect varies with the type of insect. Another explanation might be that the amounts of coffee used in the current study were lesser as [33] used 250g/L to exhibit high mortality effects.

According to this research, emulsions of *J. curcas* had a maximum of 13.3% mortality for mealy bugs [34]. Obtained similar results which showed that *J. curcas* contact toxicity on *Harmigera* larvae was too low. Low mortality of mealy bugs for the *J. curcas* treatmenst could be due to the absence of secondary metabolites in the leaves [19]. Mentioned that *J. curcas* contains a purgative oil and a phytotoxin, found mainly in the seeds and several toxic compounds such as lectin, saponin, carcinogenic phorbol, and a trypsin inhibitor which kill insects. These results also showed that emulsions from *J. curcas* compared to the other plants used, had highest repellency (72 – 92%) for mealybugs. Similar results were obtained by [35] when he used powdered parts of *J. curcas* to reduce oviposition in *Callosobruchus maculatus* on cowpea seeds in storage. The emulsion of *J. curcas* leaves at the concentration of 15g of plant powder can be safely used by low resource-poor rural farmers to discourage oviposition by adult mealybugs on banana.

In the field, the infestation of mealy bugs varied significantly between the positive control and the plant emulsions with lowest infestation for *L. camara* and then garlic. This very low infestation in the field reflects the high mortality and repulsion of these botanicals in the laboratory. Combining the emulsions of *J. Curcas* with that of either *A. sativum* or *L. camara* could increase mortality and repulsion of mealy bugs simultaneously and therefore resulting to further lower infestations in the field.

5. Conclusion

The results obtained from both laboratory and field studies demonstrated the strong potential of *Lantana camara* and *Allium sativum* emulsions, followed by *Coffea arabica* and *Jatropha curcas* emulsions for managing mealybugs. I5g *Lantana* and I0g garlic emulsions exhibited the best potency in killing mealy bugs. Some of these plants are consumed (garlic and coffee) or used by human beings as fuel (*Lantana* and physic nut) with relative safety for the environment, humans and non-target beneficial soil organisms. In addition, these plant materials are relatively abundant in the study area and the ease of producing the plant emulsions make them a viable management strategy for incorporation in the local IPM strategy to manage mealy bug infestations in banana and plantain fields.

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