Bioefficacy of vapour effect of essential oil formulation from *Syzygium aromaticum* against *Callosbruchus maculates*

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Abstract: A laboratory study was conducted to determine the efficacy of vapour effect of essential oil of clove bud *Syzygium aromaticum* using different formulations of solid carriers (silica gel, alumina and kaolin) on Bean weevil (*Callosbruchus maculatus*). The bioactivity of essential oil extracted by hydro distillation was assessed. Different masses of clove oil were measured and mixed with 1g of solid carrier; insect mortality of the vapour effect of the different formulation was evaluated. Eugenol was found to have highest percentage of composition in clove bud (95.75%). All treatments with the essential oil formulations showed significant level of toxicity to insects. The highest level of mortality in insect was recorded when 0.5g of the essential oil was mixed with 1g of solid carriers, and the result for each carriers was not significantly different.

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Key words: *Callosbruchus maculatus,*vapour, essential oil composition, *Syzygium aromaticum,* formulation.

1. Introduction

Bean weevil (*Callosbruchus maculatus*) are important pests of pulse crops in Asia and Africa under storage conditions (Ajayi and Lale 2001; and Ogunwolu and Idowu, 1994), and major post-harvest insect of beans in Nigeria (Okonkwo and Okoye, 1996), which causes huge losses to farmers during storage. In order to prevent such losses, the use of synthetic insecticides such as Phosphine-based fumigant as well as methyl bromide (MB) is used in pest control management. MB has been phased out in 2005 in developed countries and it is schedule to be phased out in the developing countries in the year 2015, because of its effect as an ozone-depleting substance (Donahaye et al, 2001).

Synthetic Insecticides have been widely developed and are extensively used because of their effectiveness and easy application, their extensive use as brought about several disadvantages, like environmental disturbances, pest recovery, pest resistance, lethal effects on non-target organisms and toxicity to users and consumers (Prakash and Rao, 1997). The use of synthetic insecticides has lead to the disappearance of the traditional know how of peasant farmers who used local aromatic plants that they introduced in their granaries with crops in order to kill insects present or to repel those coming to infest their stored products (Bocke, 2002). Due to the detrimental usage of these synthetic insecticides, there is need to look into the use of botanical insecticides, which tend to have wide spectrum of activities, readily biodegradable, less toxic to mammals, and may be selective in action and the growth of development of resistance. They may be easily or cheaply produced.

Over the past 15 years, interest in botanical insecticides has increased as a result of environmental concerns and insect populations becoming resistant to conventional chemicals. Botanical insecticides are naturally occurring insecticides that are derived from plants (Isman 2000). The insecticidal activity of essential oils and plant extracts against different stored-product pests has been evaluated (Aslan et al., 2005; Ayvaz et al., 2009; Cetin and Yanikoglu, 2006; Kim et al., 2003; Lee et al., 2003; Negahban et al., 2007; Sarac and Tunc, 1995; Shaaya et al., 1991; and Tunc et al., 2000). In spite of the wide-spread recognition that many plants possess insecticidal properties, only a handful of pest control products directly obtained from plants are in use because the commercialization of new botanicals can be hindered by a number of issues (Isman 2000). Botanicals used as insecticides presently constitute 1% of the world insecticide market (Rozman et al., 2007). Essential oils from different plant species possess ovicidal, larvicidal, and repellent properties against various insect species and are regarded as environmentally compatible pesticides (Cetin et al., 2004 and Isman 2000).

Price (1985) noted that the development to resistance to an insecticide may be due to selection of a number of behavioural traits, which causes them to avoid the toxin. In the cause of a fumigant this may be manifested as a movement away from high concentration of the gas. Effectiveness of fumigant is only one of the factors to be considered in pesticide evaluation; cost must also be taken into account. The aim of this work is to evaluate the vapour effect of essential oil of clove bud *Syzygium aromaticum* using different formulations of solid carriers on Bean weevil (*Callosbruchus maculatus*).

2. Materials and Methods

2.1 Plant material collection

Clove bud were collected from an herbal plant store in Lagos, Nigeria. The identity of the plant material was confirmed at the Herbarium in Federal University of Technology Akure, Nigeria, where the Voucher specimens were also kept.

2.2 Isolation of essential oil

The ground powder of Clove bud *Syzygium aromaticum* were subjected to hydro distillation using a modified Clevenger-type apparatus for 6 h. the condensing oils were collected in n-hexane solvent (Aldrich HPLC grade). Anhydrous sodium sulphate was used to remove water after extraction. Essential oils were stored in airtight containers at $4^{O}C$.

2.3 Breeding of Insects

The founding insect culture of Bean weevil (*Callosbruchus maculatus*) was collected in infested beans that were stored in 5-liter plastic containers at a storehouse in Lagos, Nigeria. Bean weevils were reared in growth chambers at 27 -$29^{O}C$ and relative humidity of 70-80%.Initially, 50 pairs of 1-2 day old adults were placed in a jar containing black gram seeds. The jar was sealed and a maximum of 7 days were allowed for mating and ovipositor. The parent stocks were removed and black gram seeds containing eggs was transferred to fresh black gram seeds in the breeding jar that were covered with pieces of cloth fastened with rubber bands to prevent the contamination and escape of insects. The subsequent progenies of the weevil were used for the experiment (Rahman and Talukder, 2006).

2.4 Gas chromatography and mass spectrometry

Gas chromatographic analysis was performed on an Agilent 6890 N instrument equipped with a flame ionization detector and HP-5MS (30 m × 0.25 mm × 0.25 μm) capillary column, while the essential oil components were identified on an Agilent Technologies 5973 N mass spectrometer. The GC settings were as follows: the initial oven temperature was held at $60^{O}C$ for 1 min and ramped at $10^{O}C\ min^{-1}$to $180^{O}C$ for 1 min, and then ramped at $20^{O}C\ min^{-1}$to $280^{O}C$ for 15 min. The injector temperature was maintained at $270^{O}C$. The samples (1 μL) were injected neat, with a split ratio of 1:10. The carrier gas was helium at flow rate of 1.0 mL min^{-1}. Spectra were scanned from 20 to 550 m/z at 2 scans s^{-1}. Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_8–C_{24}) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature. Component relative percentages were calculated based on GC peak areas without using correction factors.

2.5 Insecticide formulation:

Clove oil (0.1 g, 0.2 g, 0.3 g, 0.4 g and 0.5 g) was measured with a chemical balance and then mixed with 1g of silica gel to produce different formulations. The formulations were allowed to stabilize for 4 minutes. Formulations where also prepared using kaolin and Alumina.

2.6 Vapour effect of the insecticidal formulation

A Petri-dish was demarcated into two half's with the aid of a mosquito net, glue to the inner surface of the Petri-dish. Clove oil insecticidal formulations were placed in one half of the Petri-dish demarcated with a mosquito net to prevent direct contact of the formulations with the Bean weevils (*Callosbruchus maculatus*). While to the other half 20 Bean weevil were introduced 4 minutes later and then closed. Control experiments were run periodically with Silica gel, Alumina and Kaolin without essential oil. Mortality of insects was noted every five minutes. For each trial, 5 replicates were made. Data on percentage Bean weevil (*Callosbruchus maculatus*) mortality were corrected using Abbott's (1925) formula.

2.7 Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) mean values were adjusted by Duncan's Multiple Range test (Duncan 1951).

3. Results

Essential oil of clove bud from Lagos, Nigeria yielded a total of 20 compounds, eugenol representing the major component accounting for 95.75% and β-Caryophyllene constituted the second largest component accounting for 3.75%. All the remaining compounds contributed for less than 0.60%. The results of vapor effect of clove bud essential oil dust formulation using silica gel are presented in Table 1. Mortality of 13.33% was observed in one hour when 0.1g of the essential oil was used in the formulation. In 50 minutes, the same 0.2g of the essential oil formulation exhibited the same mortality rate. The use of 0.3g of essential oil formulation produced 73.33% mortality of bean weevil (*Callosbruchus maculatus*). A 100% mortality was recorded when 0.4g and 0.5g of the essential oil was in the formulation in 50 minutes and 40 minutes respectively.

Table 1: Vapour effect of Clove bud Essential oil (mixed with silica gel) on Bean weevil (*Callosbruchus maculatus*) (Values were means of three replicates +S.D; Row means followed by different letters are significantly different at P< 0.05)

Qty of oil (g)	Control	0.1	0.2	0.3	0.4	0.5
Time (min)		% of *Callosbruchus maculatus mortality*				
5	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
10	0.00a	0.00a	0.00a	0.00a	0.00a	23.33b
15	0.00a	0.00a	0.00a	0.00a	13.33b	53.33c
20	0.00a	0.00a	0.00a	0.00a	16.77b	73.33c
25	0.00a	0.00a	0.00a	23.33b	46.77c	76.77d
30	0.00a	0.00a	0.00a	30.00b	53.33c	80.00d
35	0.00a	0.00a	0.00a	36.77b	66.76c	86.77d
40	0.00a	0.00a	0.00a	60.00b	73.30c	100.00d
45	0.00a	0.00a	6.67ab	66.76b	76.77c	100.00d
50	0.00a	0.00a	13.33b	70.00c	100.00d	100.00d
55	0.00a	0.00a	16.77b	70.00c	100.00d	100.00d
60	0.00a	13.33b	26.77c	73.33d	100.00d	100.00e

Table 2 shows the result of vapor effect of clove bud essential oil formulations with kaolin as the solid carrier. From the test conducted, it was observed that the use of 0.1g of essential oil in the formulation resulted in 13.33% mortality of the bean weevil (*Callosbruchus maculatus*) in 1 hour, 23.33% mortality was observed in the same duration when 0.2g of the essential oil was used in the formulation. The use of 0.3g of the essential oil in the formulation produced 73.33% mortality in 1 hour. A 100% mortality of bean weevil (*Callosbruchus maculatus*) was recorded when 0.4g and 0.5g of the essential oil was used in the formulation in 50 minutes and 45 minutes respectively.

Vapor effect of Clove bud Essential oil (mixed with Alumina) on Bean weevil (*Callosbruchus maculatus*) is shown in Table (3). The use of 0.1 g of the essential oil as formulation gave 13.33% mortality in 1 hour, 100% mortality was observed when 0.4 g and 0.5 g of the essential oil formulations was used in the test in 50 minutes and 35 minutes respectively.

Table 2: Vapour effect of Clove bud Essential oil (mixed with Kaolin) on Bean weevil *Callosbruchus maculatus*) (Values were means of three replicates +S.D; Row means followed by different letters are significantly different at P< 0.05).

Qty of oil (g)	Control	0.1	0.2	0.3	0.4	0.5
Time (min)		% of *Callosbruchus maculatus mortality*				
5	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
10	0.00a	0.00a	0.00a	0.00a	0.00a	13.33b
15	0.00a	0.00a	0.00a	0.00a	13.33b	36.77c
20	0.00a	0.00a	0.00a	0.00a	16.77b	66.76c
25	0.00a	0.00a	0.00a	23.33b	46.77c	73.30d
30	0.00a	0.00a	0.00a	26.77b	53.33c	73.33d
35	0.00a	0.00a	0.00a	36.77b	66.76c	76.77d
40	0.00a	0.00a	0.00a	56.77b	70.00c	86.77d
45	0.00a	0.00a	6.67a	66.76b	76.77c	100.00d
50	0.00a	0.00a	13.33b	66.76c	100.00d	100.00d
55	0.00a	0.00a	16.77b	70.00c	100.00d	100.00d
60	0.00a	13.33b	23.33c	73.33d	100.00e	100.00e

Table 3: Vapor effect of Clove bud Essential oil (mixed with Alumina) on Bean weevil (*Callosbruchus maculatus*) (Values were means of three replicates +S.D; Row means followed by different letters are significantly different at P< 0.05)

Qty of oil (g)	Control	0.1	0.2	0.3	0.4	0.5
Time (min)		% of *Callosbruchus maculatus mortality*				
5	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
10	0.00a	0.00a	0.00a	0.00a	0.00a	23.33b
15	0.00a	0.00a	0.00a	0.00a	13.33b	73.33c
20	0.00a	0.00a	0.00a	13.33a	26.77b	73.33c
25	0.00a	0.00a	0.00a	23.33b	53.33c	76.77d
30	0.00a	0.00a	0.00a	36.77b	60.00c	86.77d
35	0.00a	0.00a	0.00a	40.00b	66.76c	100.00d
40	0.00a	0.00a	0.00a	56.77b	73.30c	100.00d
45	0.00a	0.00a	13.33a	76.33b	93.33c	100.00d
50	0.00a	0.00a	16.77b	76.77c	100.00d	100.00d
55	0.00a	0.00a	20.00b	76.77c	100.00d	100.00d
60	0.00a	13.33b	26.77bc	80.00d	100.00e	100.00e

4. Discussion

Potential of plants essential oils as source of insecticides has been worked out and reported with references to various pests (Sukumar et al, 1991 and Tsao et al, 2002). Sumangala and Vivek (2009) analysed clove bud essential oil form Indian, a total of 15 compounds was detected with eugenol accounting for 40.64%, Palatinol-A representing the second largest component forming 38.24% of the oil content while β-caryophyllene constituted the third abundant compound of 10.4%. Clove bud essential oil from Lagos-Nigeria recorded eugenol constituting 95.75% of the oil. Chemical elucidation of clove bud essential oil during this study indicated eugenol and β-caryophyllene as the dominant constituents of the oil. The result is in consensus with earlier reports on chemical composition of the oil from different part of the world (Gbirab and Massry, 2003; Leopard et al., 2006; Park and Shin, 2005; Raina et al., 2001; Summangala and Vivek, 2009; and Zhu et al., 1995).

The vapor effect of the clove oil formulations using Silica gel, Kaolin and Alumina as solid carriers proved to be effective in the control of bean weevil. Contact toxicity of the clove oil and the safety of this oil on non-target organism including man were discussed by (Yang et al., 2003). The use of 0.5g of the clove bud essential oil mixed with Silica gel, Alumina and Kaolin produced 100% mortality of bean weevil (*Callosbruchus maculatus*) in 40 minutes, 35 minutes and 45 minutes respectively. This shows that that the release of vapor of the essential oil dust formulations is not significantly different. Donahaye et al. (2001) stated that an insecticide formulation must be effective in terms of kill and cost, Alumina and Silica gel are both synthetic and expensive to produce; while Kaolin is natural, so it is cheap. Summangala and Vivek (2009) attributed the ovicidal and larvicidal effects of clove oils against A. albopicus to the eugenol and β-caryophyllene contain in the oil, they further observed that the leaf of clove has a higher level of biocidal activity because it has a higher eugenol constituent than the bud. Clove bud essential oil from Lagos, Nigeria has a higher eugenol content than recorded by Summangala and Vivek which could likely prove that essential oil from Lagos, Nigeria may have a higher insecticidal potency.

The present study has ascertained the potential of dust formulations of clove bud essential oil on *Callosbruchus maculatus*. Environmental feasibility and plentiful availability of the oil at reasonable market price provide and extra edge for the promotion of the Kaolin formulation into a commercial product that could make the facing out of synthetic chemicals like Methyl bromide (MB) a reality in developing countries in 2015. However, further research work for optimization of the formulation for field condition and large scale application is required to upgrade the product for commercial exploitation.

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