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The influence of commercial food grade essences on the efficacy of metaldehyde for the control of Achatina (Lissachatina) fulica (Bowdich)

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The influence of commercial food grade essences on the efficacy of metaldehyde for the control of Achatina (Lissachatina) fulica (Bowdich)

Marcus Ramdwar1*, Valerie Stoute2 and Jesse Potts1

Abstract: The highly invasive giant African snail (GAS) has been found in several agricultural areas of Trinidad. Because this snail is able to feed on many different types of crops, it is proving to be a serious threat to the economic viability of farmers in this country. The results presented in this study are the most recent of several pest control strategies attempted. Five cotton pads, soaked with 1:1 mixtures (by volume) of each of five food grade essences (Aniseed, Banana, Coconut, Lemon and Vanilla) and metaldehyde, and a sixth pad of only metaldehyde (the control), were placed at strategic distances from each other in each of five blocked patterns at various sites in each of two locations, known to be infested with GAS. The number of snail deaths was recorded for each pad and the maturity stages of snails were noted. Summary statistics, stacked horizontal bar graphs, GLM models of the total number of snail deaths and of the deaths for each maturity stage all point to the same trends. The Banana: metaldehyde preparation was the most significantly effective in killing snails of all maturities at both locations. The immature snails were much more attracted to the essences than either the neonates or the adults. Some essences were more effective at one location and others were more effective at the other location, possibly because of odours from the crops at the two locations interacting with those of the essences and either enhancing or muting the attraction of the latter.

ABOUT THE AUTHORS

Dr. Marcus Ramdwar is an assistant professor of agriculture at the University of Trinidad and Tobago (UTT). His research focus is related to food security, crop production and crop protection. His research also includes studies on farmers' groups, sustainable approaches to controlling the giant African snail and utilization of the Sargassum seaweed. Professor Valerie Stoute is a prize-winning Applied Statistician, Chemist and Social Scientist, with more than 20 years of experience in research. At UTT, Professor Stoute has guided and supervised multidisciplinary research studies conducted by students from undergraduate to doctoral levels, the most prominent of which have been in environmental studies and social research, including postal reform, entrepreneurship, agricultural studies and counselling psychology. Mr. Jesse Potts is a final year Bachelors of Science student in Crop Science and Technology at UTT.

PUBLIC INTEREST STATEMENT

The giant African snail (GAS), a native of East Africa, was discovered in Trinidad in 2008 in Diego Martin. The GAS is the second worst invasive alien species in the world. As of November 2017, the snail had infested over 18 areas and is becoming very difficult to contain and to control. The control of the GAS has been primarily by the use of metaldehyde in bait formulations. The current study evaluates the use of food grade essences (Aniseed, Banana, Coconut, Lemon and Vanilla) as an attractant adjuvant to liquid metaldehyde mixtures to increase effective mortality.
1. Introduction

The giant African snail is a highly invasive pest (Teles, Fontes, & Amaral, 2004) and it is considered globally as one of the 100 pest species with the greatest invasion potential (Lowe, Brown, Boujelas & Poorter, 2000). It has been widely documented that this species, *Achatina (Lissachatina) fulica* (Bowdich), is well adapted and distributed around the world (Thiengo, Faraco, Salgado, Cowie, & Fernandez, 2007). In October 2008, it was discovered in one location in Trinidad. By November 2017, however, this snail was known to have infested over 18 new areas, with new locations of invasion emerging regularly throughout Trinidad. The human factor is considered to be the main agent for the establishment and dispersal of this pest (De Winter, 1989). The giant African snail is an established polyphytophagous pest (Rauth & Barker, 2002) and it is reported to feed on at least 500 different types of plant species (Capinera, 2011). Given that Trinidad and Tobago is self-sufficient and the lead vegetable producer in the region (Ganapat, 2013), the presence of this pest would create a major threat to food security, not just in Trinidad and Tobago but in the region. Additionally, the giant African snail is a threat to human health since it is known to be a vector of the rat lungworm, *Angiostrongylus cantonensis*, which causes *Eosinophilic meningoencephalitis* in humans (Alicata, 1991; Provciv, Spratt & Carlisle, 2000).

Several physical, biological and chemical control strategies have been used to eradicate and manage *Achatina (Lissachatina) fulica* (Rauth & Barker, 2002). However, molluscicides are essential for the management of malacoofaunal pests in agricultural ecosystems (Jayashankar, Srirthan, & Verghese, 2013). Metaldehyde, which was introduced as a molluscicide in 1936 and was first used in slug baits in the early 1940s, is still the commonest molluscicide used (Edwards, Arancon, Vasko-Bennett, Little & Askar, 2009). Metaldehyde causes the mucus cells essential for land life for slugs and land molluscs to be destroyed permanently (Triebskorn, Christensen, & Heim, 1998; Triebskorn & Schweizer, 1990). Metaldehyde-based molluscicides cause an excessive increase of fluid excretion from the soft snail body, thereby leading to snail mortality (Kassem, Sabra, Koudsieh, & Abdallah, 1993). Metaldehyde is usually formulated as a food-based pellet and is highly toxic to domestic pets and other animals if consumed (Hollingsworth, Howe & Jarvi, 2013). Several studies (Ravikumara, Manjunatha & Pradeep, 2007; Shevale & Bedse, 2009) have evaluated the use of metaldehyde in food and food waste formulations as poison baits for the management of the giant African snail. The perishability of such formulations and their attractiveness to non-target pests would limit their overall effectiveness.

*Achatina fulica* is powered by its sense of smell (Albuquerque, Peso-Aguiar, & Assunção-Albuquerque, 2008) and as such potential substances other than perishable food-based baits could be used as attractants containing metaldehyde. Although there has been limited literature on the use of food flavouring essence as an additive to poisons or traps for crop protection initiatives, some success with these has been reported in insect control (Ogu, Ewuim, Akunne, Ononye, & Abajue, 2015; Ullah, Wardak, Badshah, Ahmad, & Kakar, 2015). The present study was conducted to investigate common food flavouring essences as a viable matrix for metaldehyde and to compare the relative effectiveness of different essence/metaldehyde combinations for the control of the giant African snail.

2. Materials and methods

2.1. Study area

The study was conducted in an abandoned ochro (*Abelmoschus esculentus*) field and a breadfruit field (*Artocarpus altiss*) in the Orange Grove farming district (10°37′25″ N 61°21′11″ W) in Trinidad. This is a flat, agricultural area, with a lot of weed vegetation as well as active crop production. The
The study was carried out on days during the rainy season but not during rainfall events. Temperatures during the study were in the range of 26–28°C, with precipitation levels at 36–38% and humidity at 90–92%.

The abandoned ochro field was overgrown with several species of grasses and a large snail population was observed. There was no treatment for controlling the snail population in the ochro field in the four months prior to this study since this field was abandoned. The breadfruit site, located approximately 600 m from the ochro field, at the point of the investigation was treated with commercial snail bait. The breadfruit field was littered with debris from fallen leaves which served as a refuge for the snails. The breadfruit field was also observed to determine snail activity prior to the investigation. A high snail population density was the pre-determining criteria for site selection to conduct the investigations.

2.2. Experimental procedure

Food grade flavouring essences were purchased from local supermarkets for use in this study. None of the labels gave any information about the constituents of the essences so that there needed to be some assessment of the actual compositions. Spectra of five food flavourings and of metaldehyde, which was used as a control, were obtained from a Gas Chromatograph Triple Quad Mass Spectrometer (GC/MS/MS), with a split/splitless injector. The instrument was fitted with a 30 m non-polar solid phase capillary column and used helium as the carrier gas. The starting oven temperature was 80°C, which was raised to 200°C, using a rate of 10°C per minute, and from there raised to 280°C at 4°C per minute. Spectra for the five original essences, metaldehyde, and the five essence:metaldehyde mixtures (1:1) are shown in Figures 1–11. The identified peaks in the spectra are given in Table 1.

Five food grade flavouring essences, Aniseed, Banana, Coconut, Lemon and dark Vanilla, were mixed in a 1:1 volume ratio with a commercial liquid formulation of 25% metaldehyde (2,4,6,8-tetramethyl-1,3,5,7-tetroxocane) diluted to a 2% solution. Circular cotton pads with an area of 19.63 cm² were saturated with each of the 1:1 essence:metaldehyde mixtures and placed into a Petri dish. There were six treatments including a control, containing only metaldehyde without any essence, and each treatment was replicated at five different sites in each of two locations. A randomized complete block design was used to place one of each of the five essences:metaldehyde treatments and the control in an extended “W” row pattern in the field. The six cotton pads were placed at the five points of the “W” and the one extended point. The extended “W” row patterns were placed 1.5 m apart and each

![Figure 1. Aniseed essence.](image-url)
extended “W” row pattern was placed 4 m apart in order to minimize intra-treatment effect. There were five extended “W” blocks randomly selected at each location for the investigation. Note that only one of each of the essence treatments is in each extended “W” so that it is the “W” blocks themselves which are replicates. The placement of the treatments into the field occurred at 5 pm and data was recorded 14 h later at 7 am the next day. The number of dead snails at each treatment location was recorded, along with the measured length of each snail. The classification for the maturity stages of the giant African snail—neonate (7–20 mm shell length), immature (20–45 mm) and adult (>45 mm)—and the definition of clinical mortality, as outlined by Ciomperlik et al. (2013), were employed in this study.
2.3. Data analysis
The means of the five “replicate” values of snail deaths for each treatment and for the control, at each of three maturities for the dead snails, were calculated at each of the two locations.

2.3.1. General linear model (GLM) analysis of treatment and location effects
GLM univariate models, without and with the maturity stage as an additional independent variable, were estimated, using SPSS V.22, for the impact variables of treatment and location, using either the total number of snail deaths as the outcome or the number killed for each of the three maturity stages at the outcome.

3. Results

3.1. Frequency distributions of snail deaths
Snail deaths for the five treatment preparations are charted according to maturity stages in Figure 12 (location 1—Orange Grove) and Figure 13 (location 2—Blue Waters). The segment lengths of
each bar are the total numbers for all snails of a particular maturity collected at the five replicate sites. Actual numbers of deaths are noted on each segment of each bar, although they are not as clear for the smaller segments. These frequencies at each maturity can be compared from one essence treatment to another and from one location to the other. The charts provide a visualization for the statistical tests (main and interaction effects) done in the GLM analysis.

Several trends can be observed from the information in Figures 12 and 13. Fewer snails seem to be killed at the Blue Waters location than at the Orange Grove Ochro field at every maturity stage (and in total) for every essence treatment. Banana/metaldehyde seems to be clearly the most effective essence preparation and more effective than the control, metaldehyde alone. After Banana, Vanilla seems to be the most effective preparation at Orange Grove but, curiously, Lemon is the second most effective preparation at the Blue Waters location. At both locations, for every treatment immature snails are the largest number killed.
3.2. Treatment means of number of snail deaths

Mean numbers of dead snails at different maturities, for each treatment at each of the two locations, the Blue Waters compound and the Ochro field, are shown in Table 2. The standard deviations were also calculated but are not included because they mask the trends between the means. They are relatively large, mainly because the “replicate” values used to get the means were not exactly measured under the same conditions but were actually in different “W” blocks, placed at different sites within each location, so any variability due to the influence of this different site placement is included in these overall measures of precision. The means in Table 2 are just used to obtain a rough view of treatment trends not to estimate statistical significance.

The statistical significance of differences in means can only be estimated within the context of the pooled variance. The test statistics in the GLM models in Section 3.3 below include the variances among these “replicates” and the p-values associated with these test statistics are used to discuss statistical significance. This is a deliberate element of the design which is used to ensure that all confounding effects from extraneous variables are captured.
in the within-group variability. Hence, any effects found to be significant are not confounded by heterogeneous densities of snails at different sampling spots within each of the two locations studied.

Clear trends can be seen in the relative mean values. Irrespective of the location or the treatment, the largest mean numbers of snails killed are those in the immature stage of maturity. The Banana: metaldehyde treatment in the ochro field stands out in every category except for the mean numbers of adults killed. At the Blue Waters Estate, however, it is only most effective for the immature snails being just marginally better than the Aniseed treatment for the adults and not as good as the Lemon preparation for the neonates. This supports much of what was seen in the stacked bar graphs in Figures 12 and 13, but again, nothing can be said about the significance of these trends, just from consideration of the means, as stated previously.
3.3. GLM models

The results are given in Table 3 for the univariate ANOVA model which estimates the significance of the impact, on the total number of snails killed, of changing treatments at different locations. The main effects of treatment and location, as well as the interaction effect of treatment × location are all significant. (The interaction plot is given in Figure 14.) The model can be fitted with an $R^2$ value of 0.643 (adjusted $R^2$ is 0.561). $R^2$ here is taken to have the same meaning as in Regression Analysis, estimating the % of the variance in the outcome variable (total snail deaths) explained by the model. It is a

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**Table 1. Compounds identified in GC-MS-MS spectra of essences used**

| Food essence spectrum | Peak retention time (min) | Chemical structure |
|-----------------------|--------------------------|--------------------|
| Metaldehyde (control) | 7.42                     | 1,3,5,7-tetroxocane, 2,4,6,8-tetramethyl (C₉H₁₆O₄) [CAS# 108-62-3] |
|                        | 8.23                     |                    |
|                        | 21.71                    |                    |
| Aniseed                | 11.33                    | Benzene, 1-methoxy-4-(1-propenyl) (C₁₀H₁₂O) [CAS#104-46-1] |
| Banana                 | 6.62                     | 5-Hepten-2-one [CAS# 110-93-0] |
|                        | 10.88                    | 2-Ceceral, (Z)- [CAS#2497-25-8] |
|                        | 12.29                    | 2-Undecenal [CAS # 2463-77-6] |
|                        | 12.74                    | ~2,4,7,9-Tetramethyl-5-decyn-4,7-diol [CAS Number 126-86-3] |
|                        | 21.70                    | Trans-9-octadecenoic acid, methyl ester [CAS # 1937-62-8] |
| Coconut                | 10.93                    | 2 (3H)-Furanone, 5-butyldihydor- [CAS#104-50-7] |
|                        | 12.89                    | Hydrocoumarin, 3,4-dihydro-1-benzopyran-2-one [CAS # 119-84-6] |
|                        | 13.68                    | Ethyl Vanillin, 3-ethoxy-4-hydroxybenzaldehyde [CAS#121-32-4] |
|                        | 15.07                    | Methyl 3-(2-hydroxyphenyl) propionate, CAS # 20349-89-7 |
| Lemon                  | 11.63                    | Cis-Limonene oxide, CAS#13837-75-7 |
| Vanilla                | 12.90                    | Vanillin, 4-hydroxy-3-methoxybenzaldehyde CAS# 121-33-5 |

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Figure 12. Distribution of dead snails at different maturity stages. The number in each category is the total from the five replicate sites in Location 1 (Orange Grove).
**Figure 13.** Distribution of dead snails at different maturity stages. The number in each category is the total from the five replicate sites in Location 2 (Blue waters).

**Table 2.** Treatment means for the number of dead snails

| Location                           | Treatments of 1:1 essence: metaldehyde | MEANS |
|------------------------------------|----------------------------------------|-------|
|                                    | All         | Neonate | Immature | Adult |
| **Orange Grove ochro field**       |             |         |           |       |
| Control                            | 18.8        | 3.0     | 11.0      | 4.8   |
| Vanilla                            | 17.8        | 2.4     | 11.4      | 4.0   |
| Coconut                            | 10.4        | 1.8     | 4.6       | 4.0   |
| Aniseed                            | 5.2         | 0.6     | 2.6       | 2.0   |
| Banana                             | 33.4        | 8.2     | 21.0      | 4.2   |
| Lemon                              | 11.8        | 3.4     | 5.2       | 3.2   |
| Total                              | 16.2        | 3.2     | 9.3       | 3.7   |
| **Blue Waters Estate**             |             |         |           |       |
| Control                            | 6.4         | 1.8     | 3.0       | 1.6   |
| Vanilla                            | 5.0         | 0.8     | 2.0       | 2.2   |
| Coconut                            | 6.0         | 0.4     | 4.4       | 1.2   |
| Aniseed                            | 10.2        | 1.2     | 4.0       | 5.0   |
| Banana                             | 23.6        | 1.4     | 15.4      | 6.8   |
| Lemon                              | 14.8        | 3.0     | 9.8       | 2.0   |
| Total                              | 11.0        | 1.4     | 6.4       | 3.1   |
| **Total**                          | 12.6        | 2.4     | 7.0       | 3.2   |
|                                    | 11.4        | 1.6     | 6.7       | 3.1   |
|                                    | 8.2         | 1.1     | 4.5       | 2.6   |
|                                    | 7.7         | 0.9     | 3.3       | 3.5   |
|                                    | 28.5        | 4.8     | 18.2      | 5.5   |
|                                    | 13.3        | 3.2     | 7.5       | 2.6   |
|                                    | 13.62       | 2.3     | 7.9       | 3.4   |
measure of goodness of fit, in that the closer the value is to 1.0, the better the fit. The relative closeness of the two $R^2$ values suggests that there is little variance inflation in the model. The adjusted unaccounted for variation (44%) is due to variability in “replication”, which may be larger than in methods with high precision because of the sample site variability, as explained above, but also may be due to missing drivers, unknown independent variables not included in the model.

More snails are killed at the Orange Grove ochro field than at the Blue Waters Estate. Post hoc tests (LSD) indicate that the only significantly different treatment from all others is the Banana preparation ($p = 0.000$ for all comparisons). The Lemon mixture has a similar impact to that of the control (metaldehyde only) but kills significantly more snails than the Aniseed ($p = 0.037$) and, at the 10% critical level, the Coconut ($p = 0.057$) mixtures.

Table 3. Univariate GLM model for different essence treatments at two locations

| Source                  | Type III sum of squares | df | Mean square | F     | Sig. |
|-------------------------|-------------------------|----|-------------|-------|------|
| Corrected model         | 4086.583$^a$            | 11 | 371.508     | 7.864 | .000 |
| Intercept               | 11,124.817              | 1  | 11,124.817  | 235.49| .000 |
| Treatment               | 2919.083                | 5  | 583.817     | 12.358| .000 |
| Location                | 410.817                 | 1  | 410.817     | 8.696 | .005 |
| Treatment × Location    | 756.683                 | 5  | 151.337     | 3.203 | .014 |
| Error                   | 2267.600                | 48 | 47.242      |       |      |
| Total                   | 17,479.000              | 60 |        |       |      |
| Corrected total         | 6354.183                | 59 |            |       |      |

$^aR^2 = .643$ (adjusted $R^2 = .561$).

Figure 14. Interaction plot for GLM univariate model (outcome = total number of snail deaths).
The treatment effect, which shows only the Banana mixture as significantly different, is clarified by the interaction plot in Figure 14. Except for Banana, which is effective at both locations, the other treatments do better at one or the other location so that when the locations are considered together in the treatment main effect, the means at the two locations balance each other out, again except for Banana, so that all the other treatments appear to be equally effective but significantly less than Banana. This is also why the interaction effect is significant. This supports what was observed in Section 3.1 and in Figures 12 and 13, when it was observed that different essences, except for Banana, performed better at one or the other location.

The results for the expanded GLM model, using Maturity stage as an additional driver, are given in Table 4. All main effects (Treatment, Location and Maturity stage), two of the three two-way effects (Treatment × Location and Treatment × Maturity stage), and the three-way effect for the Treatment × Location × Maturity stage interaction effects were significant at the 5% critical level. The interaction plots are given in Figures 14 and 15. The fit improved to $R^2 = 0.665$ (adjusted $R^2 = 0.584$). This corresponds to just 2.5% more of the variance in snail deaths explained.

**Table 4. Univariate GLM model (maturity stages) for different essence treatments at two locations**

| Source                | Type III sum of squares | df  | Mean square | $F$   | Sig. |
|-----------------------|-------------------------|-----|-------------|------|------|
| Corrected model       | 3357.128*               | 35  | 95.92       | 8.18 | .000 |
| Intercept             | 3708.272                | 1   | 3708.27     | 316.04 | .000 |
| Treatment             | 973.03                  | 5   | 194.61      | 16.59 | .000 |
| Location              | 136.94                  | 1   | 136.94      | 11.67 | .001 |
| Maturity              | 1031.88                 | 2   | 515.94      | 43.97 | .000 |
| Treatment × Location  | 252.23                  | 5   | 50.45       | 4.30  | .000 |
| Treatment × Maturity   | 606.92                  | 10  | 60.69       | 5.17  | .000 |
| Location × Maturity    | 39.74                   | 2   | 19.87       | 1.69  | .187 |
| Treatment × Location × Maturity | 316.39 | 10  | 31.64       | 2.70  | .005 |
| Error                 | 1689.60                 | 144 | 11.73       |       |      |
| Total                 | 8755.00                 | 180 |             |       |      |
| Corrected total       | 5046.73                 | 179 |             |       |      |

*$R^2 = .665$ (adjusted $R^2 = .584$).

Post hoc tests (LSD) were used to examine pair-wise differences in the means for the maturity stages. These indicated that significantly ($p = 0.000$) more immature snails were killed than either adults or neonates. The mean number of adult snails killed, however, was not significantly ($p = 0.085$) larger than that for neonates.

The interaction plots in Figure 15 are as informative here as they were for the model for total snail deaths for all maturities. These align more with the stacked horizontal bar graphs in Figures 12 and 13. They also support the results from the statistical tests. Banana was the most effective preparation for attracting snails at any maturity stage at each of the two locations. Most snail deaths occur among the juvenile/immature populations at both the Orange Grove ochro fields and
the Blue Waters Estate. For the adults and the neonates, the Control, Vanilla, Coconut, Aniseed and Lemon mixtures have essentially the same impact on the mean number of snail deaths at the Orange Grove field. At the Blue Waters Estate, however, Aniseed is more effective than the other preparations (except Banana) for the adults. For the juveniles, the patterns are also different at the two locations. For the juveniles on the other hand, at the Orange Grove field, the Control and the Vanilla mixture seem far more effective than the other essences, except for Banana. At the Blue Waters Estate, it is the Lemon mixture (along with Banana) which stands out from the rest for the juveniles, supporting the result from the LSD post hoc tests. These trends illustrate why the two-way interactions of Treatment × Location and Treatment × Maturity stage are significant. They also
illustrate why, although the overall Location × Maturity is not itself significantly different, the effect is different from one treatment to the other so that the three-way effect is significant (Table 4).

4. Discussion and conclusion

The food essences in these treatment preparations are simply attractants, bringing the snails into a targeted kill zone. It is the metaldehyde in all treatments inclusive of the control which actually causes the effective mortality. No other species, insects or animals, were observed being attracted to the traps, possibly because the essences are not food based. Similarly, it is unlikely that foraging animals, either rats or birds, depleted the numbers of dead snails before they were tallied. The dead snails were picked up before the sun rose to avoid the risk of birds consuming them. Farmers were baiting for rats in these fields so the rat populations were relatively controlled. Even if foraging animals did pick up dead or moribund snails, it is unlikely that they would have selectively picked up snails killed with a certain treatment or of a certain maturity, thereby affecting the relative frequency distributions of maturities and totals measured.

The Banana essence was found to be the best attractant at both locations. No supporting evidence was found in the literature which references the possibility that there can be preferential attraction at different stages of snail maturity. However, based on this study that may be what is happening here. More immature snails are killed, with every treatment, at both locations. This could just be representative of the relative populations of snails of different maturities at different locations and not to preferential attraction. However, one argument against this is that the Blue Waters Estate was treated closer to the start of the study than the Orange Grove ochro field, which had been left abandoned for four months. So, one would expect that Orange Grove field to have a relatively large population of adult snails. Yet the patterns are quite similar for the two locations, as seen from the totals at each stage in Figures 12 and 13 and the means in Table 2. Hence, the greater efficacy of the essence/metaldehyde treatments with immature snails seems to be a real effect.

The interaction plots in Figure 14 show that Banana is the best attractant at both locations, but for the other essences, some are more effective at the Orange Grove ochro field and others at the Blue Waters Estate so that the main effect of treatment on the total number of snails killed is significant only because Banana stands out. We suggest that the background odours from the different crops at each location (breadfruit is planted at the Blue Waters Estate) may interact differently with the essences, either enhancing the effect of some essences (Control, Vanilla, and Coconut) at one location (Blue Waters Estate) or muting the attraction at the other (Orange Grove ochro field). The post hoc LSD tests for the univariate model, with “Total Number” of snails as the outcome variable, estimate that only the Banana/metaldehyde treatment attracts and kills a significantly higher mean number of snails than is achieved with the other four essences and the control. The study concludes that Banana food grade essence has the potential to be used as an inexpensive adjuvant to liquid metaldehyde formulations to improve its performance as a molluscicide.

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Cover Image
Source: Dr. Marcus Ramdwar

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