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Studies on the Efficacy of Some Selected Botanicals against Enset Root Mealybug (*Cataenococcus ensete*) Williams and Matile-Fererro (Homptera: Pseudococcidae)

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ABSTRACT

Different botanicals were tested for their effect on enset root mealybugs' mortality in the laboratory and in pot experiments. Seed-water suspension of 10% *Millettia ferruginea* was toxic to enset root mealybugs. The dose-response bioassay of *M. ferruginea* was calculated to be $LD_{50} = 40.39$ mg/5 cm^3 of soil. With the pot experiment, drenching the soil on which the infested young enset plants were planted with seed water suspensions of 10% *M. ferruginea* caused a higher level of mortality (66%) compared to the other botanicals and the untreated plants. On the other hand, two times applications of *M. ferruginea* improved its efficacy from 66% to 79%. However, *M. ferruginea* was inferior to the synthetic insecticide Diazinon 60% EC in the pot and dipping experiments. Drenching seed-water suspension of 10% *M. ferruginea* in to the root zone of infested enset plants in the field was found to be effective against the enset root mealybugs. *Millettia* trees are abundantly found in the area and seeds can be collected and stored for long time. The preparation is simple and requires less technical skills. Thus, combinations of dipping young enset seedlings in *Millettia* solutions and drenching of the solutions to the root zone of infested enset plants can be used for the management of enset root mealybugs

Keywords: *Azadirachta indica*, *Melia azedarach*, *Millettia ferruginea*, *Phytolaca dodecandra*, *Schinus molle*

INTRODUCTION

Enset (*Ensete ventricosum*) is the most important source of dietary carbohydrate in South and South-Western Ethiopia. It serves as staple and co-staple food for more than 20 million people, providing more than 65% of the caloric requirements of the country (Brandt *et al.* 1997; Azerefegne *et al*. 2009). The underground stem (corm) and base of the pseudo stem of enset contain a large quantity of starch which serves as readily available food for human beings (Bizuayehu 2002). Apart from its use as food, enset is utilized in various ways in South and South-Western Ethiopia. High quality fiber is extracted from the leaf sheaths, home-made beer is brewed from enset crude starch, and the residues from enset processing is an important source of animal feed (Bezuneh and Feleke 1966). In the enset growing regions of Ethiopia, it is also a reliable animal feed during the dry season, when other plants are not available (Bezuneh and Feleke 1966).

However, the sustainability of enset production is highly threatened by both biotic and abiotic factors (Addis 2005). Among the biotic factors bacterial wilt and root mealybugs are the most important. Enset root mealybug, *C. ensete*, is a major pest in enset-growing areas of Ethiopia (Addis *et al.* 2008). It was first collected and reported from Wonago, Southern Ethiopia (Tsedeke 1988). It attacks any age group of enset plant but the infestation is more common on 2-4 years old plants. The problem is more serious on plants that are water stressed and grown on infertile soils (Addis 2005).

The key aspect to controlling root mealybug is prevention. However, if root mealybug infestation is well established, it is advisable to use direct control of the insect with the use of insecticides or botanicals. Some botanical insecticides which have been reported to be very effective in controlling other insect pests may also have a similar effect. The objective of this study therefore was to evaluate the efficacy and dose effects of several local botanicals on enset root mealybug mortality.

MATERIALS AND METHODS

Study area description

The trials were carried out in the laboratory and in farmers' fields. The laboratory trials were conducted at the Awassa Agricultural Research Center entomology laboratory, while the pot and field experiments were conducted on farmers' fields in Wonago, Southern Ethiopia. Wonago district is located at 1,900 masl and at 376 km south of Addis Ababa. It has an annual rainfall of 1,289-1,527 mm, while the average minimum, maximum and mean annual temperatures are 8.5, 26.7 and 17.6°C, respectively. The most common soil type across Wonago district is Nitosol. The predominant crops grown in Wonago district are enset and coffee. Fruit trees and other long lived multi-purpose trees are also present.

Botanicals collection and preparation

Seeds of *Millettia ferruginea*, *Azadirachta indica*, *Melia azedarach*, *Phytolaca dodecandra* and *Schinus molle* were evaluated for their efficacy against the enset root mealybug. Seeds of *M. ferruginea*, *M. azedarach* and *S. molle* were collected from the Gedeo area. These seeds were dried under shade and kept under a ventilated condition in the laboratory. Variety 'E44' seeds of *P. dodecandra* were obtained from the Addis Ababa Patho-biology Institute, while seeds of *A. indica* were collected from Melkaworer Agricultural Research Center, Ethiopia. All dried seeds were pul-

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verized using a mortar and chisel and were subsequently used as water suspensions in the different experiments.

Petri dish bioassay

Water suspensions of each botanical were prepared by soaking the grounded seeds at the rate of 10 g/100 ml of water. This was an ideal suspension to do subsequent filtering using a muslin cloth. The mixtures were kept for 24 h and subsequently filtered using muslin cloth. Accordingly, a 10% concentration of aqueous suspensions was used for the different tests. In order to obtain large numbers of root mealy bugs, enset plants infested with root mealybugs were collected the day before the experiment from the Gedeo area, south Ethiopia and taken to the laboratory. Adult mealybugs collected from the roots and corms of these plants were carefully detached and kept on Petri dishes with some soil. Ten adult mealybugs were placed in each Petri dish (9 cm diameter) with 5 cm^3 soil and drenched with 4 ml of the respective botanicals (i.e. *M. ferruginea*, *S. molle*, *M. azedarach*, *P. dodecandra* and *A. indica* for seed-water extraction respectively). Each treatment was replicated three times. Data on mealybug mortality was collected during three days at 24-h intervals. Dead mealybugs were counted and removed every day and the cumulative mortality percentage was calculated**.**

Pot experiment - drenching

Two-years-old infested enset seedlings of the cultivar 'Genticha', were collected from Gedeo, southern Ethiopia. 30 adult mealybugs were kept on each seedling. The enset seedlings were transplanted in plastic polybags of 40 cm diameter and 30 cm height filled with soil and with holes on their underside to avoid water logging. The potted enset plants were kept in an enset farm. For each botanical treatment (i.e. seed water solution of *M. ferruginea*, *S. molle*, *M. azedarach*, *P. dodecandra* and *A. indica*) six potted enset plants were used and each plant was considered as a replicate. The botanicals were prepared following the same procedure as described for the Petri dish experiment. The botanical suspensions were drenched to the root collar of the seedlings at a rate of 990 ml in each plastic bag. Diazinon 60% EC diluted at a rate of 1: 500 with an application volume of 990 ml per bag was used as the standard check. The control plants received similar amounts of water.

Data on mortality of the mealybugs was collected after a week by uprooting all the seedlings. Live adult mealybugs were counted and recorded and percent mortality for the respective treatments was calculated.

Field trials - dipping

Two year old enset plants of the cultivar 'Genticha', each having a total of 40 mealybugs on the corm and roots were used for the different treatment. The prepared seed water suspensions (as for the Petri dish and pot experiments) at 10% concentration (w/v) were used. The root and root collar regions of five seedlings which were allocated to each treatment were kept submerged for three minutes in 10 l of the respective botanical solution, the standard check (Diazinon 60% EC) and the control (pure water). The seedlings were subsequently planted out in the field under a completely randomized design in an area where enset plants had not been planted before. The seedlings were uprooted after 10 days and the number of surviving mealybugs was counted.

Dose response bioassay

For the most effective botanical, *M. ferruginea*, different concentration response bioassays were conducted by preparing aqueous suspension concentrations of 0.1, 0.5, 1, 3, 5, 7 and 10%. To obtain the different suspension concentrations, the 10% (w/v) aqueous suspension was further diluted in accordance with the respective treatment concentrations (i.e., 0.1, 0.5, 1, 3, 5 and 7%). Diazinon 60% EC was used as the standard check and was diluted at 1: 250 l in water as recommended by the manufacturer. The control consisted of pure water. The same protocols as for the Petri dish bioassays were used.

Data analysis

Data collected from all experiments were analyzed using the GenStat software (GenStat 2003). The percentage of mealy bug mortality was corrected according to Abbott (1925). Data for mortality percentage were arcsine transformed before ANOVA analysis. Means of percent mortality for the various treatments were separated using Tukey's HSD test at $P<0.05$.

RESULTS

Petri dish bioassay

Amongst the different botanicals tested for their toxicity against enset root mealybugs, *Millettia ferruginea* (Hochst) resulted in significantly higher levels of mortality compared to the other botanicals (**Table 1**). *A. indica*, known to have insecticidal properties against many insects was the least effective amongst the tested botanicals. Three days after treatment, *M. azedarach*, *P. dodecandra*, and *S. molle* caused a 24, 29 and 33% mortality, respectively.

Pot experiment - drenching

M. ferruginea caused 66% mortality when applied on infested enset plant in pots, which was significantly higher than the other botanicals (**Table 2**). It was observed that, one application was not enough and repeated applications were necessary to obtain higher levels of mortality.

When the *M. ferruginea* seed water suspension was applied twice at an interval of one week on potted enset plants a significantly higher level of mortality (79 ± 1.44%) was obtained compared to the single application (66.1 ± 2.04%) (**Fig. 1**). The additional application improved the mortality by 13%.

However, the efficacy of *M. ferruginea* was significantly lower than the commercial insecticide Diazinon ($P<0.05$). While *M. ferruginea* caused 66% mortality, Diazinon caused 99% mortality (**Table 2**). Low levels of enset

Table 1 Toxicity of botanicals to enset root mealybug in a laboratory (Petri dish) experiment.

Treatments (4 ml/5 cm^3 of soil in the Petri dish)	Mortality (%) hours after treatment					
	24 h		48 h		72 h	
	Obs.*	Cor.	Obs.	Cor.	Obs.	Cor.
Azadirachta indica	16.7 c	10.11	20.0 cd	13.41	36.90 a	19.62
Melia azedarach	16.6 c	10.11	23.3 c	13.41	33.33 a	23.72
Phytolaca dodecandra	10.0 c	3.40	23.3 c	13.41	40.00 a	29.03
Schinus molle	13.3 c	6.70	36.6 b	26.73	43.30 c	33.03
Millettia ferruginea	80.0 b	73.42	100.0 a	90.02	-	-
Diazinon 60% EC	100.0 a	93.45	-	-	-	-
Control	6.6 d	-	9.9 d	-	10.0 b	-
CV (%)	16.6	-	12.4	-	16.19	-

Means followed by the same letter in a column are not significantly different from each other; according to Tukey's HSD test, P<0.05
*: Obs. = Observed mortality, Cor. = Corrected mortality

Table 2 Toxicity of botanicals to enset root mealybugs in potted enset plants.

Treatments	n*	Mortality (%) observed	Mortality (%) corrected	Number of live mealybugs /plant
S. molle	35	18.1 ± 2.29 c	4.11	25.8 ±5.2
A. indica	32	18.1 ± 2.76 c	4.11	25.8 ± 6.5
P. dodecandra	34	18.5 ± 1.91c	4.5	25.2 ± 6.7
M. azedarach	32	21.5 ± 2.90 c	7.5	25.0 ± 4.3
M. ferruginea	32	66.1 ± 2.04 b	52.17	10.8 ± 3.5
Diazinon 60 % EC	32	99.1 ± 0.00 a	85.22	0.3 ± 0.0
Control	32	14.0 ± 2.57 c	-	27.5
CV (%)	-	18.4	-	-

Means followed by the same letter in a column are not significantly different from each other; according to Tukey's HSD test, P<0.05
*: n = mean number of enset root mealybugs/plant before treatment

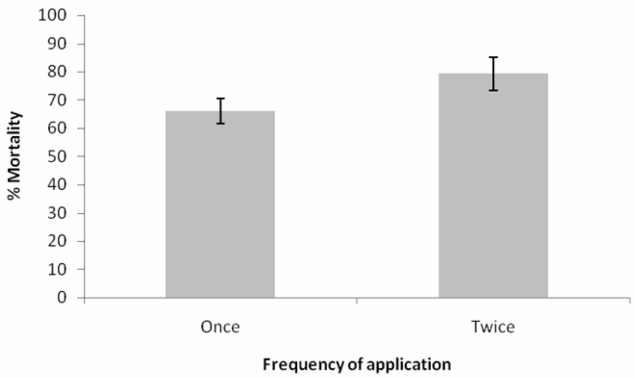

Fig. 1 Extent of enset root mealybug mortality when infested enset plants were treated once or twice with a *M. ferruginea* seed water suspension (t-test, P<0.05).

Table 3 Mortality of enset root mealybugs due to dipping of infested enset seedlings in various seed water extracts.

Treatments	Observed mortality (%) after 10 days	Corrected mortality (%)
P. dodecandra	11.7 ± 3.1 c	5.2
S. molle	12.7 ± 0.8 c	6.2
A. indica	12.5 ± 1.2 c	6
M. azedarach	17.6 ± 3.0 c	11.1
M. ferruginea	44.2 ± 2.1 b	37.9
Diazinon 60 % EC.	78.3 ± 3.5 a	72.2
Control	6.5 ± 0.6 c	-
CV (%)	25.7	-

Means followed by the same letter in a column are not significantly different from each other; according to Tukey's HSD test, P<0.05

Table 4 Toxicity of different concentrations of *M. ferruginea* seed water suspensions to enset root mealybugs in a Petri-dish experiment.

Concentration (g/100 ml of water)	% mortality (hours after treatment)					
	24 h		48 h		72 h	
	Obs.*	Cor.	Obs.	Cor.	Obs.	Cor.
10	80.00 b	80	100 a	97	-	-
7	63 c	63	70 b	66	86 a	82
5	53 c	53	63 b	60	66 b	63
3	26 c	26	30 c	27	37 c	33
1	3 d	3	6 d	2.7	19 d	15
0.5	3 d	3	6 d	2.7	9 d	6
0.1	0 d	0	3 d	0	6.6 d	3.4
Control	0 d	-	3 d	-	3.3 d	-
Diazinon 60 % EC	100 a	100	-	-	-	-
CV (%)	24.8	-	13.9	-	11.9	-

Means followed by the same letter within a column are not significantly different; according to Tukey's HSD test, P<0.05
*: Obs. = Observed mortality, Cor. = Corrected mortality

root mealybug mortality were recorded for the other four botanicals and these were not significantly different from the untreated control.

Field trials - Dipping

Dipping of root mealybug infested enset seedlings in the various botanical solutions did not result in significant mortality except for the 10% concentration of *M. ferruginea* (**Table 3**). The other four botanicals were not significantly different from the untreated control. *M. ferruginea* was however inferior compared to the commercial insecticide Diazinon (Ethiozinon) produced by Adamitulu Pesticides Processing S.CO (**Table 3**).

Dose-response bioassay

The 5% *M. ferruginea* solution resulted in a 66% mortality within 72 h (**Table 4**). A strong positive relationship ($y = 1.2354x – 1.4621$; $R^2 = 0.99$) was observed between the log concentration of *M. ferruginea* and the probit kill (**Fig. 2**).

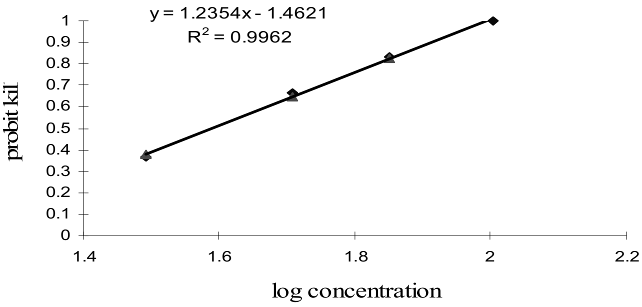

Fig. 2 Regression of dose of seed-water suspension of *M. ferruginea* and mortality of enset root mealybugs.

The effective dose to kill 50% of the population (LD_{50}) was calculated using probit analysis and it was found to be 40.39 mg while the LD_{90} was 77.62 mg.

DISCUSSION

Among the five botanicals tested, only the seed-water suspension of *M. ferruginea* resulted in a high mortality of mealybugs suggesting that it can be useful in controlling mealybug infestations. However, in the pot experiment *M. ferruginea* resulted in a relatively low mortality of 66%. The results suggest that one application is not sufficient and several applications are needed.

On the other hand, dipping infested enset plants into an *M. ferruginea* solution resulted in lower levels of mortality (44.2%) compared to drenching (66.1%). Prolonging the dipping duration could increase enset root mealy bug mortality levels. It would be advisable to combine the dipping treatment with additional periodic drenching for a better control of the pest.

Leaf, bark and seed of *M. ferruginea* have been reported to have insecticidal properties. For example, *M. ferruginea* seed powder extracts resulted in a 96% mortality rate of maize weevils (*Sitophilus zeamais*) 72 days after treatment (Bekele 2002). Over 25 flavonoids, 50 isoflavonoids, 12 chalcones and other miscellaneous compounds have been produced from the *Millettia* genus alone (Bekele 1988). Rotenone is one of the dominant compounds found in the seed and stem bark of *M. ferruginea* (George 1980; Bekele 1988). It is a powerful inhibitor of cellular respiration, the process of converting cell nutrients into energy (George 1980). It acts primarily on insects' nerve and muscle cells, causing the insects to stop feeding. Insect death occurs from several hours to a few days after contact.

Rotenone at a 1 and 5% concentration has been used against the Colorado potato beetle when the pest became resistant to all government-approved synthetic insecticides in the USA (Kuepper 2003). Rotenone is a broad-spectrum contact compound effective against leaf-feeding insects, such as aphids, certain beetles (e.g., asparagus beetle, bean leaf beetle, Colorado potato beetle, cucumber beetle, flea beetle and the strawberry leaf beetle) and caterpillars, as well as fleas and lice on animals (Buss and Park-Brown 2008).

M. ferruginea is a big perennial tree reaching up to 35m height. The plant is endemic to Ethiopia and is widely distributed across the country. The tree grows well in moist lowlands as well as in dry, moist and wet semi-highland agro-climatic zones (1,000-2,500 masl) (Azene and Brinie 1993). It is the most abundant and dominant component of the agro-forestry system of the study area where it is extensively used as shade for coffee. A single tree can produce large amounts of seeds and is thus easily available to resource poor farmers.

Results from the study indicate that seed-water extracts of *M. ferruginea* are highly toxic to enset root mealybugs compared with the other four botanicals. Dipping young enset seedlings in a *Millettia* solution before field establishment and subsequent multiple drenching (at least two times) of the root zone of infested enset plants with the solution can be used for the management of the enset root mealybug.

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