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In vitro Evaluation of PHYTOSANEB-020, A Biopesticide for the Control of Mealybug (*Planococcus ficus*) on *Rosa multiflora* and *Bougainvillea spectabilis* in Kimwenza-Mission

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study focused on the *in vitro* evaluation of the effectiveness of Phytosaneb-020, a bio-pesticide on Mealybug *Planococcus ficus* (Signoret) which infests *Rosa multiflora* (*Rosa polyantha*) and *Bougainvillea spectabilis*. Three treatments were used: Phytosaneb-020, Pacha a chemical insecticide and aqueous solution (distilled water) as a negative control. A volume of 1.5 mL for an area of 56.72 cm^2 corresponding to the area of a Petri dish, used for each treatment. Populations of *Maconellicoccus hirsutus* were carefully collected from bougainvillea leaves and roses. Pacha gave the highest mortality percentage for direct toxicity by contact (98.3± 2.89%) followed by Saneb-020 (93.3 ± 7.64%). The negative control, distilled water, was not effective against the insects. No death was observed (0%). The repellency effect assay revealed a repellent activity of 80% ± 20 for Saneb-20. Distilled water gave the best avoidance effect (86.7±11.6%) pointing out

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the fact that Mealybug *Planococcus ficus* doesn't support the presence of water. Pasha exhibited low repellency effect on Mealybug *Planococcus ficus* (46.7±41.6%). Further investigations on different pests are strongly suggested and encouraged to improve the effectiveness of this biopesticide.

Keywords: Mealybug; Rosa multiflora; Bougainvillea spectabilis; Phytosaneb-020; Biopesticide.

1. INTRODUCTION

Mealybugs (Hemiptera: *Pseudococcidae*) are small, soft-bodied, plant sucking insects which embrace the second largest family of scale insects (*Pseudococcidae*) and comprises approximately 2000 species belonging to 300 genera[1]. These insects cause major economic losses by attacking a wide variety of crops, fruits, vegetables, ornamentals and weeds but cotton is the prime target [2]. At the initial stage the damage of the insect appears in small pockets and then spreads to whole field. This pest has two common names (pink mealybug and hibiscus mealybug). Among mealybug species, *Planococcus ficus* (Signoret) is considered key pest of *Rosa multiflora* (*Rosa polyantha*) and *Bougainvillea spectabilis* causing serious damages on these ornamental plants [3].

Fig. 1. The species *Rosa multiflora* (*Rosa polyantha*) and *Bougainvillea spectabilis* attacked by *Planococcus ficus* (Signoret)

Ornamental crops of Rosa multiflora (*Rosa polyantha*) and Bougainvillea spectabilis suffer the invasions of Mealybug Planococcus ficus (Signoret), which prevent the good progress of photosynthesis in the leaf, by the secretion of honeydew, infecting these leaves with sooty mold virus. In fact, *Planococcus ficus* feeds on the soft tissues of many plant species and injects a toxic saliva that causes curling and contortion of leaves. Therefore, the entire plant may be stunted and the shoot tips develop a bushy appearance. Buds may not flower and stems may twist. Fruit may also be deformed. The mealybug excretes honeydew which encourages the development of black sooty mold. Very high mealybug populations can kill plants [4-5].

The level of feeding damage depends on the vigor of the infested plant; seedling trees and weakened trees are more susceptible. Shoots become twisted with shortened internodes, forming bunchy heads of small bushy leaves at the tips. The curled leaves can resemble viral damage, but this pest is not known to vector any diseases. Heavy infestations of young plants by Mealybug *Planococcus ficus* may stunt their growth [6].

Biopesticides offer a great promise in controlling yield loss without compromising the quality of the product. In fact, biopesticides have several advantages over their chemical counterparts and are expected to occupy a large share of the market in the coming period. Biopesticides are natural, biologically occurring compounds that are used to control various agricultural pests infesting plants in forests, gardens, farmlands, etc. In contrary, chemical pesticides pose many long-term threats and risks to living beings due to their harmful side effects. They are known to cause cancers and foetal impairments and they persist in the environment for many years because some of them are nonbiodegradable [7-10].

According to Komivi Senyo Akutse and co-workers [11], in Africa, biopesticide use is still at its infancy and only accounts for 3% of the world biopesticide market. A little information is accessible on adoption rates of biopesticides on the continent. Kenya and South Africa are leading in biopesticide development and use. In USA, the marketability of essential oils, represent a market estimated at USD 700.00 million and a total world production of 45,000 tons. Thus, there is real need to promote research in Africa in this particular area of Agriculture.

Plant derivatives can be used as an alternative approach to chemical pesticides [12-13].

The main objective of this study was to evaluate, under laboratory conditions, the toxicity of Phytosaneb-020 (Fig. 2), a bio-pesticide manufactured by Groupe de genies Congolais (GGC) at the Bioenergies laboratory of the Faculty of Science and Technology of Loyola University of Congo (ULC) on Mealybug *Planococcus ficus* populations (adults, nymphs, and eggs).

Fig. 2. Phytosaneb-020

2. MATERIALS AND METHODS

2.1 Study Site

This study was conducted in the Laboratory of Bionergies of Faculty of sciences et technologies at Université Loyola du Congo(ULC) in Kinshasa, Democratic Republic of Congo from April to June 2015.

2.2 Insect Rearing

We collected the insects from of *Rosa multiflora* (*Rosa polyantha*) and *Bougainvillea spectabilis* near the Main Library of the University. We used pruning shears to cut off the offshoots of the plants carrying the insects and placing them on a tray and leading them into our laboratory. We collected a total of up to 400 insects (adults, nymphs, and eggs) for our entire experiment. Harvest took place about 30 minutes before the application of treatments in the morning at around 9-10 a.m [14]. Bioassays were carried out in the laboratory, with an average temperature of 28 ± 1°C. All the experiments were performed in triplicate.

2.3 Preparation of the Treatment Solutions

2.3.1 Saneb-020

Chemical composition: essential oils of five plants: *Allium sativum* (L.), *Capsicum annuum, Zingiber officinale, Cymbopogon citratus and Eucalyptus obliqua.* 21 mL of each essential oil were mixed in container and dilluted with distilled water (20% Ethanol) up to 5 Liters. This stock solution was later used for experiment.

2.3.2 Pacha

PACHA, a broad-spectrum foliar insecticide that can be used as a preventative or curative was used as positive control. Chemical composition: Lambda-cyhalothrine (15 g/L), EC IRAC: 3A and Acetamipride (10g/L), EC IRAC: 4A. Following the indication established by the manufacturer(1L/ha), 1 mL of Pacha was mixed with 299.82 mL of distilled water before spraying on a surface of 56.72 cm^2.

2.3.3 Distilled water

Distilled water was used as a negative control.

2.4 Contact Toxicity

The toxicity by contact of the treatments was investigated with the method proposed by Z. L. Liu and S. H. Ho [15]. Arenas consisted of Petri dishes. 1.5 mL of each treatment (Saneb-020, Pacha and distilled water) was uniformly spread on a filter paper disk (56,72 cm^2). The filter paper was dried in air for 15 minutes to allow the solvent to evaporate before putting into Petri dish. After we proceeded by counting the number of mealybugs and forming populations of 10 insects per petri dish and respecting the homogeneity of the populations and the petri dish was covered. All tests were carried out in three replicates and the count of dead insects was determined after 1 hour.

2.5 Repellent Activity

We used the methodology described by Mvenga and Lokadi with few modifications [16]. The repellency effect was evaluated using the preferential zone method on filter paper. The 8.5 cm diameter filter paper discs used were cut into two equal parts each having a surface area of 28.36 cm^2. A volume of 1.5 ml of each treatment was spread on a half of the disc while the other half received no treatment. After 15 minutes, the time required for complete evaporation, the two halves of the discs were placed in a Petri dish and a batch of 10 adult insects were placed in the center and the petri dishes were closed. After 1 hour, the number of insects on the portion of the filter paper treated was recorded. The percentage of avoidance was calculated using the formula below:

$$\mathrm{PR} = \frac{\mathrm{STD-SNT}}{STD+SNT} \times 100 \ (1)$$

STD= Surface treated
SNT=Surface non treated
PR= repulsion percentage

The average percentage of repulsion for each treatment was determined according to the classification made by McDonald *et al.* [17].

2.6 Statistical Analysis

All data generated were subjected to analysis of variance (ANOVA)using Excel 2015 and the significant mean differences ($p > 0.05$) were separated by using the student-Newman-Keuls (SNK) test.

3. RESULTS AND DISCUSSION

3.1 Contact Toxicity Test

The results as recorded in Table 1 show the mortality rate observed after one hour of direct contact between different treatments and insects. Pacha gave the highest mortality percentage (98.3± 2.89%) followed by Saneb-020 (93.3± 7.64%). The negative control, distilled water, was not effective against the insects. No death was observed (0%) (Fig. 3).

The chemical pesticide Pacha and Saneb-020 have exhibited approximately the same potential to kill the Mealybug *Planococcus ficus* populations in one hour.

3.3 Test on the Repellent Effect

The repellent effect of each treatment was recorded after 1 hour. According to Table 2, a repellency effect of 80.0±20.0% was observed. Pasha exhibited low repellency effect on Mealybug *Planococcus ficus* (46.7±41.6%). The best avoidance effect was observed with distillated water (86.7±11.6%). According to the classification of Mc Donald *et al*., Pacha could be classified in class III (40.1 - 60%), Saneb-020 in class IV and distilled water in class V [17]. In other words, Mealybug *Planococcus ficus* doesn't support the presence of H_20 or SANEB-020 compare to Pacha (Fig. 4).

The results obtained from this research could be an indication of the effectiveness of the biological insecticide Saneb-020 as a candidate for the control of Mealybug (*Planococcus ficus*) on *Rosa multiflora* and *Bougainvillea spectabilis*. In fact, Saneb-020 has exhibited a good direct toxicity with a mortality of 93.3% a little low compare to the chemical insecticide Pacha, 98.3% in one hour of exposure.

Table 1. Contact toxicity assay

Treatments	Number of Insects	Number of deaths (Mean ± STD)	%
Pacha	20	19.67±0.58	98.3
Saneb-020	20	18.67±1.53	93.3
H_20	20	0	0

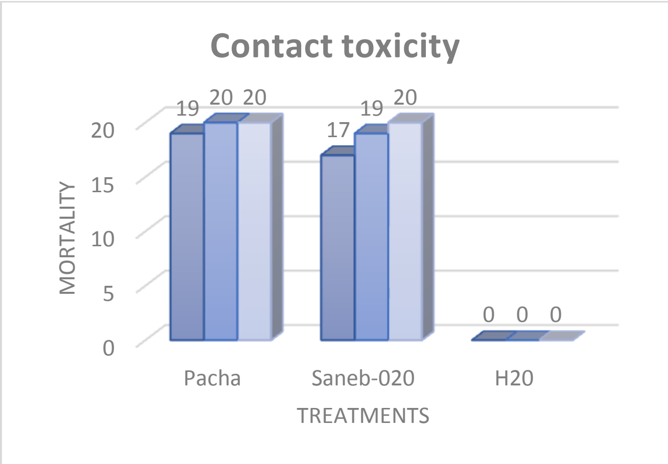

Fig. 3. Histograms of mortality observed after one hour of direct contact with Mealybug *Planococcus ficus* populations

Table 2. Repellent effect assay

Treatments	Effectif of Insects	Surface treated (STD)	Surface non treated (SNT)	Repulsion percentage (%)
Pacha	10	7.3±2.1	2.7±2.1	46.7±41.6
Saneb-020	10	9.0±1.0	1.5±1	80.0±20.0
H_20	10	9.3±0.6	0.7±0.6	86.7±11.6

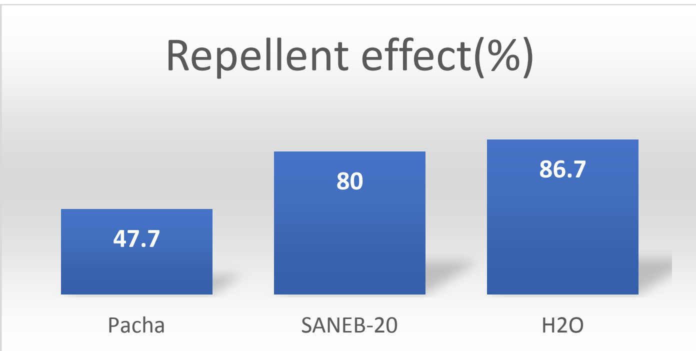

Fig. 4. *In vitro* repellent effect of treatments on Mealybug *Planococcus ficus* after one hour

Saneb-20 exhibited also a good repellent effect on Mealybug (*Planococcus ficus*) with an average rate of avoidance of 80% ± 20 which means that this bio insecticide has a medium insect repellent activity according to the classification of McDonald *et al*. [17]. This result shows clearly that Saneb-020 has a stronger insect repellent activity than Pasha on Mealybug (*Planococcus ficus*). This could be due to the combination of bioactive molecules contained in essential oils used in the manufacturing of Saneb-020 including allicin, gingerol, capsaicin, citral and cineole. Plant extracts and essential oils exhibit a wide range of action against insects: they can act as repellents, attractants, or antifeedants; they also may inhibit respiration, hamper the identification of host plants by insects, inhibit oviposition and decrease adult emergence by ovicidal and larvicidal effects [18-20].

In fact, according to Nwachukwu and Asawalam [21], the freshly prepared garlic (*Allium sativum* L.) juice, containing allicin, was evaluated as a possible grain pro- tectant against the maize weevil, *Sitophilus zeamais* (Motsch.) and exhibited lethal effects causing at least 90% adult mortality in contact toxicity tests. Compounds such as allicin lead to suffocation of the pest due to effects on receptors of neurotransmitters [12]. Capsaicin can act as a deterrent to affect animal behaviors, such as egg laying choice. In 2020, Yaoxing Li et co-workers reported that *Drosophila* females exhibit a robust ovipositional aversion to capsaicin. They found that females were robustly repelled from laying eggs on capsaicin-containing sites [22].

Insecticidal and repellent activity of essential oils of *Zingiber officinale* and *Eucalyptus globulus* against *Culex theileri* Theobald were evaluated and the result showed considerable values of insecticidal and repellent activity against mosquitoes, concentration of one (undiluted extract) had the highest insecticidal and repellent activity against *Culex theileri Theobald* for both of essential oils and essential oil of Eucalyptus globulus (66% insecticidal and 74% repellent activity) was more potent than Zingiber officinale (45% insecticidal and 61% repellent activity. [23]. Dry powders of ginger (*Zingiber afficinale*), hail (*Elettaria cardamomum*) and shammar (*Foeniculum vulgare*) were tested for their toxicity against the adult beetle *Oryzaephilus surinamensis*, as date pest threatens the date product in Saudi Arabia. All the tested plants showed insecticidal activity against *O. surinamensis*. Ginger is the most potent plant, recording the lowest LC_{50} value (0.14 mg/g) followed by hail and shammar (LC_{50} = 0.4 and 0.7 mg/g) respectively [24]. Sumitra and colleagues (2014) evaluated a biopesticide formulation containing onion (*Allium cepa*) and ginger (*Zingiber officinale*) against tomato fruit worm (*Helicoverpa armigera*) and they registered a 70% - 80% control [25].

In 2018, Lengai and Muthomi reported that in vitro experiments involving several ethanolic plant extracts such as turmeric (*Curcuma longa*), lemon (*Citrus limon*), garlic (*Allium sativum*), pepper (*Capsicum frutescens*) and ginger (*Zingiber officinale*) were reported to be significantly effective against Alternaria solani, Pythium ultimum, Rhizoctonia solani and *Fusarium oxysporum f. sp. Lycopersici* [26].

Development of biopesticides in Africa is still in the early stages [27]. In this regards, SANEB-20 could be a good, efficient and safe alternative to chemical pesticides because it is ecological and easy to manufacture [28-32]. In addition, it could help to control the yield loss caused mainly by Mealybugs (Hemiptera: *Pseudococcidae*).

4. CONCLUSION

The effectiveness of a biopesticide, Saneb-020 was assessed under laboratory conditions on Mealybug (*Planococcus ficus*) which infects *Rosa multiflora* and *Bougainvillea spectabilis*. The results obtained showed clearly that the combination of active molecules contained in Saneb-020 has a strong insecticidal activity causing a mortality of 93.3% in 1 hour of direct contact the insects. The repellent activity (80% ± 20) exhibited by this biological insecticide confirm its effectiveness against the *Planococcus ficus*. Further investigations on other insects are strongly recommended in order to promote this biological insecticide.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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