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Biological control using essential oil of *Ocimum gratissimum* and four other biopesticides on *Formicococcus njalensis*, the most active mealybugs species in the transmission of CSSV (*Cocoa swollen shoot virus*)

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A laboratory investigation was conducted to evaluate the use of *Ocimum gratissimum* essential oil to control the development of *Formicococcus njalensis*, the main vector of *Cocoa Swollen shoot virus* (CSSV) in West Africa. Commercial biopesticides (*Squad*, *Agriphy*, *Mycotal* and *Banole*), the essential oil of *O. gratissimum* obtained by hydrodistillation were compared at the concentration of 0, 1000, 2000 and 3000 µl.l^{-1} respectively. Ten mealybugs were directly sprayed using the different concentrations. The mortality rate of the mealybugs was evaluated by counting the number of mealybugs dead in each Petri dish 24, 48, 72 and 96 h after treatment. The highest toxicity was observed with Squad which killed all the mealybugs at each of the 3 concentrations. The second highest toxicity was observed with *O. gratissimum* treatment which mortality rate increased with the concentration. Indeed, a mortality rate of 39.27, 50 and 59% was observed at 1000, 2000 and 3000 µl.l^{-1} respectively. The third highest lethality rate was observed with Agriphy (29.36%) at 1000 µl.l^{-1} and with Banole (48%) at 2000 µl.l^{-1}. At 2000 µl.l^{-1}, Agriphy and Mycotal lethality rates were 41.4 and 5% respectively. No death was observed in the control treatment. Conclusively, environmental friendly biopesticides such as *O. gratissimum* and Squad can be used to control *Formicococcus njalensis* population, and hence limit CSSV prevalence.

Key words: Cocoa swollen shoot disease (CSSD), mealybugs, biological control, *Ocimum gratissimum.*

INTRODUCTION

The cocoa swollen shoot disease (CSSD), a vector born transmitted disease, is considered as the most important threat in West Africa due to its decreasing effects on yield and causing mortality of on infected cocoa plants when severe strains occurred (Aka et al., 2020; Jones, 2020). This disease is characterized by specific symptoms

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including foliar red vein-banding, fern mosaic pattern on old leaves, stems and roots swellings with tree decline and death 3-5 years after symptoms development (Domfeh et al., 2019; Kouakou et al., 2011; Ramos-Sobrinho et al., 2020). The CSSD is caused by at least ten species viruses belong to *Badnavirus* genus including CSSV and over 20 species of mealybugs are involved in the spreading of the disease in field through a semi-persistent manner (Roivainen, 1976; Ramos-Sobrinho et al., 2020). The control of this disease based on cutting out of infected plants and replantation using improve resistant planting materials are facing to many challenges including, using monocropping system (Naturland, 2014), reinfection on new replant farms (author) , expansion of the disease on many cocoa grown areas and the lack of the efficient biopesticides to combat the mealybugs vectors (M. Peschiutta et al., 2018). Synthetic products failed to combat mealybugs. This is because mealybugs have an important waxy cover which protects them from most synthetic insecticides. The dispersal of nymphs between the interlocking canopies of adjacent trees probably spread locally CSSV. Also, mealybugs are well protected from sun, natural enemies and from most foliar insecticides because they hide under bark or in the roots of plants. Mealybugs are cottony in appearance, small, oval, soft-bodied sucking insect. Adult mealybugs are found on leaves, stems and roots and are covered with white mealy wax, which makes them difficult to eradicate. They form colonies on stems and leaves developing into dense waxy, white masses. They suck a large amount of sap from leaves and stems with the help of piercing/ sucking mouth parts, depriving plants of essential nutrients. The excess sap is excreted as honedew which attracts ants and develops sooty mould inhibiting the plant's ability to manufacture food (Tanwar et al., 2007). Traditional mealybugs control uses synthetic insecticides that have undesirable effects on environmental and human health. It is necessary to develop environmentally friendly pesticides from natural products for *Formicococcus njalensis* controls have not yet reviewed. Consequently, the aim of this work was to revise the current knowledge about organic products used to protect cocoa, in order to identify the main limitations in mealybugs management and to propose new strategies to control this pest. Thus, essential oils (EO) are considered as an interesting alternative to synthetic pesticides because of their efficiency and versatility. Indeed, their volatility and chemical diversity make them excellent fumigants, insecticides and repellents (Sousa et al., 2015). Consumer demand for naturally active compounds such as EO has stimulated the search for new products that reduce or completely replace the use of synthetic insecticides that are harmful to human health and the environment (Peschiutta et al., 2017). This is particularly important for the control of mealybugs, as their body is covered with a waxy substance that prevents the entry of traditional insecticides, while the EO are lipophilic compounds that penetrate the body of the insect and exert their toxic effects (Ahmed, 2016). Therefore, the objective of this study was to evaluate the insecticidal properties of *O. gratissimum* and 4 biopesticides against *Formicococcus njalensis* by direct contact in petri dishes.

EXPERIMENTAL APPROACHES

Collection of *O. gratissimum* leaves and essential oil extraction

Leaves of *Ocimum gratissimum* were collected in the field of producers installed by the Plant Physiology Laboratory in Gonzagueville (Abidjan). Only healthy young shoots were harvested to obtain better qualities of essential oil. These leaves were placed in the open-air room and protected from light for one day. Then stored in paper bags and shipped to the laboratory for the extraction of the essential oil. The collected leaves were used to extract the essential oil by hydrodistillation with the Clevenger device for 3 h. The extracted oil was stored in dark bottle and put in refrigerate at 4°C. *O. gratissimum* extracts also possess an important biocidal effect (i.e. insecticide, repellent, anti-pest) on various pests such as malaria vectors, stored food pests and field crops (Ikele et al., 2020; Yarou et al., 2018).

Mealybugs collection

The mealybugs (*Formicococcus njalensis* (Planococcoidae) were collected in infected cocoa orchards with apparent disease symptoms in the village of Petit Bouake, Soubré (South West Côte d'Ivoire). A small brush was used to loosen the mealybugs attached to the different organs of the cocoa tree. Adults mealybugs were collected because they easier to collect than juvenile ones. A sharp needle was used to collect mealybugs firmly attached to the organs. The mealybugs were the acclimatized in the laboratory by exposing them on cocoa leaves overnight. All the mealybugs collected survived to the acclimatization.

Contact toxicity bioassay through direct spraying

The experiment was done using 9 mm diameter's Petri dishes. For each concentration, 6 Petri dishes were used. Concentrations 0, 1000, 2000 and 3000 ppm were used. The preparation was done using ethanol and distilled water to ensure the miscibility of essential oil as recommended by the World Health Organization (WHO). A moisture filter paper (Whatman N°1) was laid in the petri dish. Pieces of young cocoa leave sprayed with the different treatment (biopesticides at the different concentrations) was put on the filter in the corresponding, petri dish. Each treatment was replicated three time. A laboratory precision sprayer and the spray pressure was adjusted to between 54 and 80 kPa pressure (Ahmadi et al., 2012) was used. A total of ten mealybugs were the released on each Petri dish. The toxicity level biopesticides was performed by counting the alive population of mealybugs after 24, 48, 72 and 96 h respectively. The mealybugs were considered dead if the appendages donot move. This was then confirmed by incising them with a fine-bristle brush under microscopic observation (Choi et al., 2003). The insecticide percentage was calculated according to Singh et al. (2012):

$$PI = [(NC – NT) / (NC + NT)] \times 100$$

Where: PI= Percentage of insecticide or Percentage of Lethality
NC = Number of Mealybugs'
NT = Insect number present on the strip treated with the extract.

Data analysis

The lethality was the independent variable and the concentrations, the time of incubation and the different extracts were dependant variables during this assess. The data were subjected to an analysis of variance (ANOVA), using R software version 3.6.1 to compare the significance at the 5% level. Newmann and Keuls' test was then used separate in the case of a significant difference ($P < 0.05$).

RESULTS

The result was shown at 24, 48, 72 and 96 h. At the concentration C1 (1000 µl/l), after 24 h of treatment of the products (*Ocimum gratissimum*, Squad, Agriphy, Mycotal, Banole and control), mealybugs did not kill any mealybug in the Petri dishes (0%). This result was the same at the other concentrations after the first day of treatment. After 48 h or 2 days of treatment at the C1, it was only the Squad that had the highest toxicity product. It killed the half (50%) of mealybugs in Petri dishes. The other products did not kill any insects after this second day on treatment. However, *Ocimum gratissimum* prorogued the oviposition of seven mealybugs on thirty. After the same level of concentration after 72 h or three days, the highest toxicity product was noticed from Squad (81,19 ± 1,3%) followed by *Ocimum gratissimum* (19.39 ± 1.59%), Agriphy (13.88±1.19%) Banole (1.85 ± 0.65%), Mycotal (1.75 ± 0.56%) and finally in the control dishes, all insects were alive (Table 1).

After 96 h of treatment, the highest toxicity was Squad where all insects died in the Petri dishes. *O. gratissimum* killed almost the third party of the mealybugs (39.27 ± 1.6%), followed by Agriphy (29.36±1.5%), mycotal (4.16± 0.6%), Banole (1.85 ±1,6%) and the control where mealybugs were alive. At the concentration C2 (2000 µl/l), after 48 h of treatment, the Squad killed almost the half party of mealybugs (50±1.73%) and then, the other products did not kill any mealybugs at this time (Table 1).\

At the same concentration after 72 h or 3 days of treatment, the best product was Squad. It caused 81.19±1.3% of mortality of mealybugs, followed by *O. gratissimum* which killed the half party of insects (50±1.73%), Banole (34.59± 1.56%), Agriphy which killed the quarter party of insects (25±1.58%). Mycotal and the control did not kill any insects in their Petri dishes (0%). After 96 h or 4 days of treatment at the same concentration (2000 µl/l) the highest product was Squad. All mealybugs died (100%) in the Petri dishes. *O. gratissimum* killed half party of insects in the Petri dishes (50±1.7%), followed by Banole (48.14±1.6%), Agriphy (41.41±1.6%), Mycotal (5.04±10% and the control where the insects were alive (Table 2). At the third concentration (3000 µl/l), after 2 days of treatment, the highest product was still Squad (50±1.73%), followed by Mycotal which killed in the average, 1 mealybug (0.98) on 10 mealybugs. The other products did not kill any insects in their Petri dishes. After 72 h Squad killed almost mealybugs (93.93±0,97% in the Petri dishes, followed by O. *gratissimum* (50±1.73%), Agriphy (50±1.73%), Banole (18.61±1.24%) and Mycotal (11.43±1.43%). In the last time (96H), the highest product was Squad which killed all the insects (100%), followed by *O. gratissimum* (58.95±1.6%), Agriphy (50± 1.73%), Banole (33.93±1.5%), Mycotal (18.25±1.30%) and the control with 0% of mortality (Table 3).

DISCUSSION

The purpose of our study is to evaluate the efficacity of each product used to control the mealybug *Formoccocus njalensis*, *Swollen shoot virus*' vector of cocoa. The use of plant extracts in the control of pests and pathogens is a long-standing practice in agriculture around the world and is highly recommended for the protection of the environment (Gurjar et al., 2012). The efficacy of botanical extracts in controlling scale insects has been tested in lot of studies (Maheswari and Govindaiah, 2018; Ahmadi et al., 2012; Prishanthini and Vinobaba, 2014; Singh et al., 2012). This assessment demonstrated the toxicity effect of *Ocimum gratissimum*'s essential oil on this vector, in Côte d'Ivoire. However, at the different concentrations of the essential oil, the first action was the negative oviposition of mealybugs at the second day. Our results about *Ocimum gratissimum* oviposition deterring effect confirm those of previous studies. For instance, *O. basilicum* ethanolic extracts negatively influenced the oviposition behaviour of another leafminer *Phthorimaea operculella* Zell. (Lepidoptera: Gelechiidae) (Sharaby et al., 2009). *Ocimum* spp. essential oils were previously shown to reduce the oviposition behaviour of *Agrotis ipsilon* H. (Lepidoptera: Noctuidae) on cotton plants (Shadia et al., 2007). Moreover, *O. gratissimum*, *O. basilicum* and O. *sanctum* L. extracts or EOs were found to have a repellent and oviposition deterrent effect on the insect pests (Dryophthoridae, Curcuilionidae, Bostrichidae, Tenebrionidae, Bruchidae) of various stored products (Asawalam et al., 2008; Kiradoo and Srivastava, 2010). Bioassays results in Petri dishes showed that *O. gratissimum* essential oil conserved its insecticidal activity to eliminate mealybugs from 50% to 58.95% despite all mealybugs did not die after the 4^{th} day of application. Hence, our result is inadequate with the one of Nguemtchouin et al. (2015). Their results showed thatO. *gratissimum* essential oil quickly lost its insecticidal activity despite being impregnated on maize to kill *Sitophilus zeamais*.

Squad is a 100% plant-based triglyceride adjuvant. This biocontrol product is used to optimize the effectiveness of biocontrol solutions and agrochemical solutions that are herbicides, fungicides or insecticides. It can be combined with microorganisms and nematodes used in biocontrol, with contact or systemic insecticides, especially on crops with tight foliage (upright growth habit, organs that are difficult to reach such as leeks,

Table 1. Mortality of mealybugs at the first day after treatment.

Products	Concentration		
	C1 (1000 µl/l)	C2 (2000 µl/l)	C3 (3000 µl/l)
Agriphy	0^{c}	0^{c}	0^{c}
Banole	0^{c}	0^{c}	0^{c}
Ocimum	0^{c}	0^{c}	0^{c}
Mycotal	0^{c}	0^{c}	0.98 ± 0.33^{b}
Squad	50 ± 1.73^{a}	50 ± 1.73^{a}	50 ± 1.73^{a}
Control	0^{c}	0^{c}	0^{c}

Table 2. Mortality of mealybugs at the first day after treatment.

Products	Concentrations		
	C1 (1000 µl/l)	C2 (2000 µl/l)	C3 (3000 µl/l)
Agriphy	13.88 ± 1.19^{c}	25 ± 1.58^{c}	50 ± 1.73^{bc}
Mycotal	1.75 ± 0.56^{c}	0^{c}	11.43 ± 1.43^{c}
Banole	1.85 ± 0.65^{c}	34.59 ± 1.56^{c}	18.61 ± 1.24^{c}
Ocimum	19.39 ± 1.59^{c}	50 ± 1.73^{bc}	50 ± 1.73^{bc}
Squad	81.19 ± 1.3^{b}	81.19 ± 1.3^{b}	$93.93 \pm 0{,}97^{a}$
Control	0^{c}	0^{c}	0^{c}

Table 3. Mortality of mealybugs at the third day after treatment.

Products	Concentrations		
	C1 (1000 µl/l)	C2 (2000 µl/l)	C3 (3000 µl/l)
Agriphy	29.36 ± 1.5^{d}	41.41 ± 1.6^{cd}	50 ± 1.7^{c}
Mycotal	4.16 ± 1.0^{f}	5.04 ± 1.0^{f}	$18.25 \pm 1{,}3^{e}$
Banole	$1.85 \pm 0{,}6^{f}$	48.14 ± 1.6^{c}	33.92 ± 1.5^{cd}
Ocimum	39.27 ± 1.6^{cd}	50 ± 1.7^{c}	58.95 ± 1.6^{b}
Squad	100^{a}	100^{a}	100^{a}
control	0^{f}	0^{f}	0^{f}

onions and leafy vegetables), with contact or systemic fungicides, especially those used against diseases of crops that are difficult to wet (cruciferous plants, legumes, cereals and grasses), but also with contact or systemic, total or selective herbicides. In our assessment, Squad was used alone without another product or essential oil. It killed 100% of scale insects treated with 1000 ppm, 2000 ppm and 3000 ppm. Our results corroborate the one of Fanou et al. (2014) which showed Cydim Super neutralized *Dysmicoccus brevipes*, the mealybugs of pineapple in Benin. The combination of the two products, *Ocimum gratissimum* and Squad, could give farmers a good insecticide to control mealybugs of cocoa.

Conclusion

The obtained results indicated that Squad eliminated *Formicococcus njalensis* after spraying, followed by essential oil of *O. gratissimum* eliminated 59% of these insects. In addition, *O. gratissimum* influenced the oviposition of *Formicococcus njalensis* after one day of spraying and neutralized all nymphs. *Formicococcus njalensis* exposed to direct contact with Mycotal, Agriphy and Banole behaved in an almost similar way as those exposed to the untreated control, showing no antifeeding or antiappetitive response. Consequently, Squad and Essential oil of *O. gratissimum* could be tested further in the nursery and field test.

CONFLICT OF INTERESTS

The author has not declared any conflict of interest.

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