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ORIGINAL ARTICLE

Bio-insecticidal effects of essential oil nano-emulsion of *Lippia multiflora* Mold. on major cabbage pests

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Abstract

The present study investigated the potential use of the nano-emulsion of *Lippia multiflora* Mold. essential oil in managing the cabbage pest (*Brassica oleracea* L.) in two Ivorian areas (Yamoussoukro and Korhogo) during the wet seasons (April-September 2018). The nano-emulsion was tested against cabbage diamondback moth (*Plutella xylostella*), aphid (*Brevicoryne brassicae)*, webworm (*Hellula undalis*), cutworm (*Spodoptera exigua*) and whitefly (*Bemisia tabaci*) under field conditions. The efficacy of essential oil emulsion was compared with the synthetic pesticide Karate 5 EC (Lambda cyhalothrin 52 g · l^{-1}). The results indicated that the nano-emulsion of essential oil gave better control of the cabbage insect pest than the untreated plots. For all the insects studied, the nano-emulsion was very effective towards the species *B. brassicae* and *P. xylostella* for which the reduction of the mean population was respectively, 28.48 ± 0.2 and 0.6 ± 0.02 in Yamoussoukro and 0.0 and 7.11 ± 0.16 in Korhogo, compared to 45.32 ± 0.43 and 15.89 ± 0.23, respectively, for untreated plots. The yields of cabbage heads obtained in both areas Yamoussoukro and Korhogo were 4.7 and 15, respectively. The head damage percentages were 23.3% in Yamoussoukro and 26.7% in Korhogo when the fields were sprayed with nano-emulsion and were 13.3% and 28.3%, respectively, when the cabbages were treated with the synthetic pesticide. The formulation obtained here might be an interesting alternative for integrated pest management of cabbage.

Keywords: biopesticide, cabbage, essential oil, *Lippia multiflora,* nanoformulation

Introduction

Cabbage is a very important vegetable produced in Africa and is consumed by both rural and urban populations. This vegetable is commonly prepared in a number of ways for eating and most frequently, it is included either as a cooked or raw part of many salads (Baidoo and Adam 2012; Baidoo and Mochiah 2016). However, cabbage is susceptible to insect pest infestations in the field, which causes huge losses to growers (Baidoo and Adam 2012). The major insect pests infesting cabbages in Africa which cause the greatest damage in the tropics and subtropics, include the diamondback moth (*Plutella xylostella*), the cabbage webworm (*Helula undalis*), the cabbage white butterfly (*Pieris brassicae*), the cabbage aphid (*Brevicoryne brassicae*), the cabbage looper (*Trichoplusia ni*) and green peach aphids (*Myzus persicae*) (Baidoo and Adam 2012; Baidoo and Mochiah 2016). These insect pests infest the *Brassica oleracea* crop at different stages of growth, causing extensive destruction to the cabbage crop during the growth stages (Furlong *et al.* 2013) and result in huge yield losses in the field.

Increasing cabbage production by enhancing productivity per unit area seems impossible without effective insect pest management. The common strategy adopted by farmers for insect pest management in cabbage crops and other vegetable crops is the use of synthetic insecticides. Ideally, insecticides should be toxic only to the target pest, biodegradable and non-persistent. However, the use of synthetic insecticides does not meet the above requirements (Gill and Garg 2014). The use of synthetic insecticides which is still intensive causes a development of pest resistance and some undesirable effects not only to the agricultural ecosystem but also to human health due to insecticide residue in food, namely in agricultural products, particularly in vegetables (Dadang *et al.* 2011).

Food safety issues have attracted global attention due to the direct consequences of conventional insecticide contamination on human health. It is imperative to restore consumer confidence in the safety of food commodities, especially those that are often eaten raw or partially cooked such as cabbage. Therefore, it is important to reduce the dependency of famers on synthetic products in cabbage crops. This purpose should be achieved by means of strategies such as the use of biopesticides.

Aromatic plants such as *Lippia multiflora* are considered to be a safe way of controlling pests (Boulogne *et al.* 2012). Scientifically, the essential oil from this plant has been reported to have insecticidal (Tia *et al.* 2013, 2020) and pesticidal properties against body lice as well as significant antimicrobial activity (Baba *et al.* 1997; Owolabi *et al.* 2009; Bassolé *et al.* 2010). Oladimeji *et al.* (2000) reported pediculocidal and scabicidal properties of the essential oil of *L. multiflora*. This oil has demonstrated promising larvicidal (Bassolé *et al.* 2003) and repellent (Tia *et al.* 2020) activities against *Anopheles gambiae*. A significant insecticidal activity against the cabbage insect pest *Bemisia tabaci* was obtained with this essential oil (Tia *et al.* 2011).

In vitro and *in vivo* results show unambiguously that all the essential oils tested possess biological activity. However, the main problem in using plant essential oils such as biopesticides under field conditions is their chemical instability in the presence of air, light, moisture, and high temperatures which lead to rapid evaporation and degradation of their active constituents (Mustafa and Hussein 2020). In addition, their water insolubility and oxidation, constitute the factors that influence essential oil activity, application and persistence under field conditions. To overcome these problems, many nanotechnological based strategies have been proposed as alternatives, especially liposomes, nano-emulsions and nanoparticles (Zorzi *et al.* 2015). Various formulations have been proposed, each one having their own advantages and disadvantages. Nano-emulsions for example, protect essential oils from degradation and increase their residue half-life by reducing evaporation. They can achieve a controlled release of essential oil and ease the application and handling (Feng *et al.* 2018). Nano-emulsions are stable for a long time, but their stability depends on the individual system or mixture of ingredients, low viscosity and droplet size.

In this current work, a nano-emulsion of essential oil and hydrolate from *L. multiflora* was made in the presence of a natural surfactant (chitosan). A mixing technique was used to obtain a nano-emulsion. This study aimed to investigate the insecticidal activity of *Lippia* essential oil nano-emulsion in managing a cabbage insect pest on a real scale. A comparative study between the insecticidal effect of the nano-emulsion and conventional insecticide, Karate 5 EC, was carried out.

Materials and Methods

Study sites

The field experiments were carried out at the experimental station of Félix Houphouët Boigny National Polytechnic Institute of Yamoussoukro, a city situated in the central zone of Côte d'Ivoire. The field is located at 6°49'14"N; 5°16'36"W. The rainfall is of double maxima type occurring from April to July and August to October with average monthly temperatures between 20°C and 35°C and an average annual rainfall of 900 mm to 11,000 mm. The second experimental field was at Nahoulakaha, near Korhogo in northern Côte d'Ivoire. This field is located at 9°27'29"N; 5°37'47"W. The northern region of Côte d'Ivoire has an equatorial and soudano-sahelien climate with an average monthly temperature between 28°C and 36°C and an average annual rainfall of 600 to 900 mm. The rainfall is of the double maxima type occurring from April to July and September to October. The dry season, which is dominated by dry harmattan winds, extends from December to February.

Essential oil and hydrolate extraction

The *L. multiflora* plants used as a source of essential oil came from Korhogo (northern Côte d'Ivoire). Essential oil was obtained by steam distillation. Fresh leaves of the plant were harvested in bags, taken to the laboratory where they were air dried at 28 ± 2°C. Fractions of dried plant material (7 kg) was submitted to steam distillation using an extractor worker 250 l (Albrigi Luigi sarl, Italy), stainless steel alembic still type apparatus for 3 h (Tia *et al.* 2011). Essential oils were then separated after decantation, while the remaining product of oil extraction was the hydrolate. Essential oil was stored in amber glass vials at 4°C until use.

Preparation of nano-emulsion

Nano-emulsion was obtained by a low energy method (Mustafa and Hussein 2020) using 10% of essential oil, 89.75% of hydrolate and 0.25% of chitosan (France kitine) according to the following steps. The hydrolate and chitosan were stirred at 600 rpm using a magnetic stirrer (RCT Basic 20 l IKA, France) for 30 min. Then, essential oil was added drop wise at a flow rate of 1 ml · min^{-1}. The mixture was stirred at 600 rpm for 60 min. The nano-emulsion was stored at room temperature (25 ± 2°C).

Experimental design, treatment application and data recording

A randomized complete block design was used with three replications in each of the two sites. There were three treatments including: control (untreated: T0), Karate 5 EC (Lambda cyhalothrin) (positive control: T1) and nano-emulsion (T2). Karate 5 EC and nano-emulsion were applied at the fixed dose of 1 l · ha^{-1}.

A knapsack sprayer (OSATU) with a capacity of 16 l was used to apply each of the treatments. Treatments were done weekly and started 2 weeks after transplanting of seedlings and lasted for 2 months. Data collection started 2 weeks after transplanting and was done on a weekly basis for all the growing sites. Insect populations were counted and recorded from the middle row excluding the border plants. A total of an average of 10 plants per plot was used for yield and insect damage assessment. These field experiments were carried out during the wet seasons (April–September 2018).

Insect sampling

Ten plants in the inner rows were carefully examined by searching the leaf surfaces and by gently searching the underside of cabbage leaves to count and record the populations of different insects observed.

Harvesting of cabbage heads for yield

The cabbage was harvested 2 months post treatment, 1 week after the last treatment application. Ten cabbage plants from the inner rows were harvested by cutting the cabbage heads from each plot. The cabbage heads were weighed using a Salter balance and the weights (kg) were recorded.

Yield quality/crop health

Crop health was evaluated based on the damage on the harvested heads. Cabbage head damage was assessed by using a standard scoring scale of 0–5 (Aboagye 1996): 0 – no head damage, 1 – 1–15% head damage, 2 – 15–30% head damage, 3 – 30–45% head damage, 4 – 45–60% head damage and 5 – 60–100% head damage, and their percentages were calculated for each treatment according to the next relation (Munthali and Tshegofatso 2014):

$$\%\ \text{Damage} = \frac{\text{Total number of damaged heads}}{\text{Total number of heads sampled}} \times 100.$$

Statistical analysis

Mean and standard error of insect pests, insect damage and the yield of cabbage were calculated and then analyzed using analysis of variance (ANOVA). Where significant differences existed, the mean separation was done using LSD at 5% significant level. Data from these experiments were analyzed using STATISTICA 7.1.

Results

Insect fauna found on cabbage field during the growing season

Some insect species were recorded during the entire cabbage growing season. These included diamondback moths (*P. xylostella*), cabbage aphids (*B. brassicae),* cabbage webworms (*H. undalis*), and cutworms (*S. exigua*) for the two fields studied. In addition, *B. tabaci* (whiteflies) were observed in Korhogo.

Insecticidal effects of nano-emulsion and conventional insecticide

Figure 1 shows the effects of the control or untreat (T0), the nano-emulsion or botanical insecticide (T2) and the positive control, conventional chemical insecticide (Karate 5 EC) (T1) on the mean population of major insect pests observed in the field experiment in Yamoussoukro. The results indicated that there were significant differences between treatments in the aphid infestation. The conventional insecticide, Karate 5 EC provided a more effective treatment with an aphid number of 2.7 ± 0.2, followed by the nano-emulsion, for which the average aphid population was evaluated at 28.48 ± 0.2. These two treatments were much better than the control

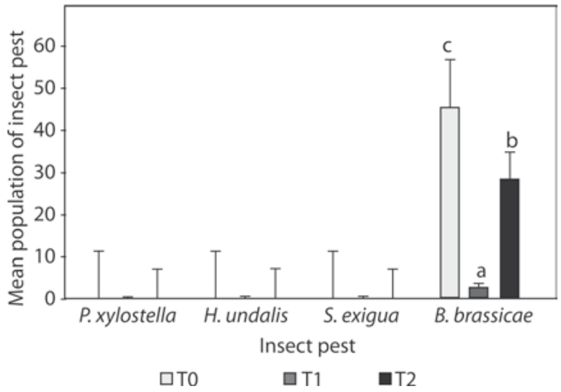

Fig. 1. Mean (±SE) of *Plutella xylostella*, *Hellula undalis*, *Spodoptera exigua* and *Brevicoryne brassicae* on cabbage sprayed with nano-emulsion or botanical insecticide (T2) and conventional chemical insecticide (Karate 5 EC) (T1) and control or untreat (T0) in Yamoussoukro. The averages of population of each insect with the same letter are not significantly different, at the 5% threshold

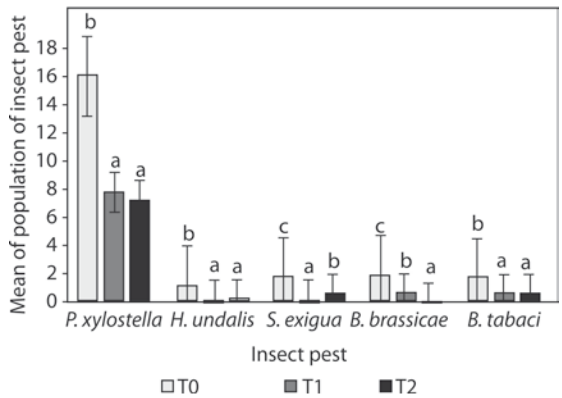

Fig. 2. Mean (±SE) of *Plutella xylostella*, *Hellula undalis*, *Spodoptera exigua*, *Brevicoryne brassicae* and *Bemisia tabaci* on cabbage sprayed with nano-emulsion or botanical insecticide (T2) and conventional chemical insecticide (Karate 5 EC) (T1) and control or untreat (T0) in Korhogo. The averages of population of each insect with the same letter are not significantly different, at the 5% threshold

(T0 treatment), for which the number of aphids was 45.32 ± 0.43.

Infestation by diamondback moths (*P. xylostella*) was high compared with other pests in the wet season in Korhogo (Fig. 2). The results showed that there were no significant differences between the treatments with Karate 5 EC (T1) and with nano-emulsion (T2) toward insect pests such as *P. xylostella*, *H. undalis* and *B. tabaci*. The nano-emulsion and Karate 5 EC had a significantly lower number of *P. xylostella* (7.11 ± 0.16 and 7.67 ± 0.18), *H. undalis* (0.22 ± 0.02 and 0.11 ± 0.03), and *B. tabaci* (0.56 ± 0.02 and 0.56 ± 0.09) than the untreated plots (15.89 ± 0.23; 1.11 ± 0.05; 1.67 ± 0.05), respectively (Fig. 2). The result also showed that there was significantly better efficacy of nano-emulsion against *B. brassicae* than Karate 5 EC.

Effects of treatments on yield and cabbage weight

The treatments made with the nano-emulsion and Karate 5 EC provided approximatively the same number of marketable acceptablity heads in both experimental fields, 15 ± 1.5 and 14 ± 1.2 in Korhogo, then 4.7 ± 1.0 and 5.8 ± 1.06 in Yamoussoukro, respectively. These values were higher than those obtained when the plots were untreated. The cabbage head weights obtained were similar for both treatments on the two growing sites (Fig. 3).

Effects of treatment on cabbage head damage

Cabbage heads were classified into marketable and unmarketable according to the number of holes per head. The severity of damage on cabbage heads on treated

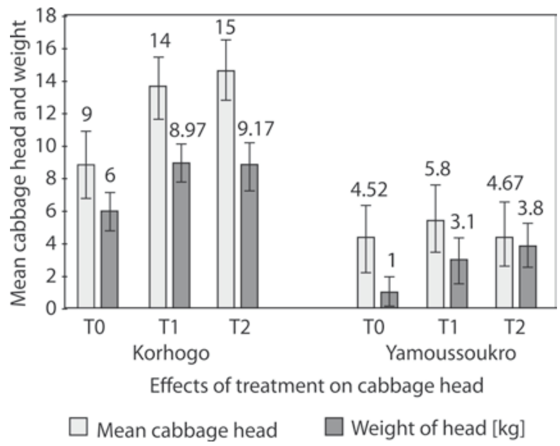

Fig. 3. Mean yield and weight of cabbage heads under different spray treatments in both regions [nano-emulsion or botanical insecticide (T2); conventional chemical insecticide (Karate 5 EC) (T1) and control or untreat (T0)]

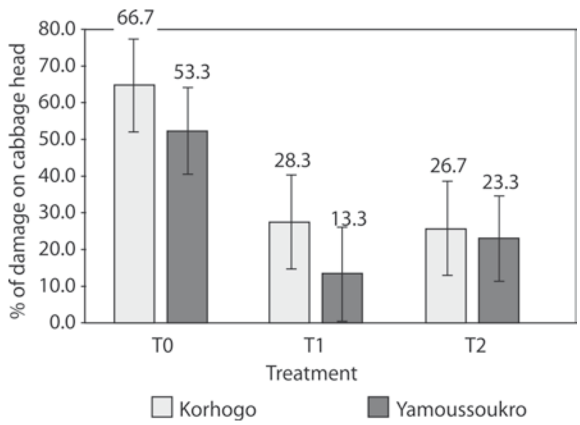

Fig. 4. The intensity of damage on the cabbage heads per treatment: nano-emulsion or botanical insecticide (T2); conventional chemical insecticide (Karate 5 EC) (T1) and control or untreat (T0)

plots was lower than on the untreated plots in both regions (Fig. 4). There were no significant differences in the head damage between the treatments in Korhogo. The damage on plots treated with Karate 5 EC and nano-emulsion was within the damage 2 (15–30%) category. On the other hand, there were significant differences in the mean head damage between treatments in Yamoussoukro. The cabbage heads from Karate 5 EC treated plots had the lowest damage (damage ≤15%) as compared to nano-emulsion which had the damage 2 (15–30%) category.

Most of the damage on the untreated plots was within the damage 4 (45–60%) and damage 5 (above 60%) categories for both regions. Both management strategies (T1 and T2), carried out in this study, provided significantly more marketable heads than the control (T0) in both regions.

Discussion

Lippia multiflora essential oil was used as a biopesticide to control insects. However, the difficulty in applying essential oils on a large-scale and under environmental conditions required incorporation of these plant materials into new formulations through nanotechnology. Nano-emulsion, for example, could enhance the efficacy, increase stability, and prevent rapid evaporation of active compounds in plant oils. The low energy method was used to develop a nano-emulsion base essential oil of *L. multiflora*. Nano-emulsion containing essential oil, hydrolate from *L. multiflora* and chitosan presented a fine, milky white appearance, which is in accordance with this type of formulation. No signs of instability, including creaming or phase separation, were observed.

The goal of the current study was to evaluate the efficacy of the nano-emulsion of *L. multiflora* as a potential botanical insecticide in providing an acceptable cabbage pest control in middle and northern Côte d'Ivoire. In both experimental areas, all materials tested resulted in the production of marketable cabbage heads with considerably lower pest pressure and crop damage ratings than untreated control plots which produced the lowest number of marketable heads. These results indicate that both treatments were effective in controlling cabbage insect pests such as *B. brassicae*, *S. exigua*, *H. undalis*, *P. xylostella* and *B. tabaci*.

The presence and abundance of insects differed from region to region. Insect pests sampled on the cabbage field in Yamoussoukro were fewer than those present in Korhogo. Whiteflies, *B. tabaci* were not found on the treatment plots on the Yamoussoukro site. This may be attributed to seasonal and climatic differences between the two areas. Indeed, climatic factors play an important role. For example, rainfall washes the eggs, larvae, pupae and the adults from the plant to the soil where they are destroyed. This may lead to the disruption of the lifecycle of the pests (Ezena *et al.* 2016).

The effectiveness of nano-emulsion in this study was generally equivalent to that of the conventional synthetic insecticide, Karate 5 EC in managing the major cabbage insect pests.

Similarly, positive results regarding insect pest reduction using nano-encapsulated plant-based insecticides in vegetable crop pest control have been demonstrated. Thus, Christofoli *et al.* (2015) found insecticidal potential of the free and encapsulated essential oil from *Z. rhoifolium* leaves against *B. tabaci*. For instance, the *Eucalyptus globulus* extract encapsulation has been successfully used to control infestations of *Myzus persicae* (Khoshraftar *et al.* 2019). Additionally, the efficacy of nano-formulations based on essential oils from various plant species has been shown. Maji *et al.* (2007) reported the repellent activity of encapsulated essential oil of *Zanthoxylum limonella* against mosquitoes. In a similar study, Solomon *et al.* (2012) reported the prolongation of the repellent effect of microencapsulation of citronella oil (*Cymbopogon nardus*) against mosquitoes. Meanwhile, the encapsulation of garlic essential oil using ethylene glycol exhibited insecticidal activity against *Tribolium castaneum* adults, improving the control efficacy of these pests in stored products (Yang *et al.* 2009). Similarly, Paula *et al.* (2010) reported that the larvicidal effect of *Lippia sidoides* essential oil nano-emulsion formulation against *Anopheles aegypti* was increased.

Despite the large number of essential oils tested as insecticides, only a few studies have focused on vegetable crops in the field. In general, very few studies on crop systems are available where both the efficacy and the impact of the insecticide treatments on the plant have been assessed in the field. In addition, most papers tend to report the acute toxicity or repellence of a given essential oil or nano-formulation in trials *in vitro*, whereas the long-lasting effects of these compounds are lacking. To the best of our knowledge, this is the first study where the efficacy and the impact of a nano-emulsion treatment on cabbage insect pests were investigated in an experimental field.

A higher yield of cabbage heads was recorded on treated plots than on untreated plots. Nano-emulsion treated plots recorded the highest mean weight of cabbage heads due to its insecticidal ability. This result confirms previous findings of other studies that have reported the efficacy of botanical treatments against cabbage insects. For example, Cerda *et al.* (2019), found that extracts from Amazon plants could significantly reduce the populations of *P. xylostella* and *B. brassicae* (Shiberu and Negeri 2016). In a similar study, the extracts of *Azadiracta indica* (neem) have been successfully used to control infestations of cabbage insect pests (Mondedji *et al.* 2014; Ezena *et al*. 2016).

The study showed that *L. multiflora* essential oil-based nano-emulsion formulation was able to reduce the mean population rate of cabbage insect pests. In this context, the nano-emulsion can be considered to be a promising insecticidal agent. This nanoformulation is eco-friendly and could further enhance the performance of these natural compounds, reducing both the dosages required and their toxicity towards non-target organisms.

Conclusions

The present study examined a nano-emulsion with insecticide activity against major cabbage insect pests. A low energy method was used to develop a nano-emulsion based essential oil, hydrolate from *L. multiflora* and chitosan. No instability was observed in this nano-emulsion.

The results of this study provide compelling evidence that nano-emulsion with essential oils from *L. multiflora* leaves optimized the control system against *P. xylostella*, *H. undalis*, *B. brassicae* and *B. tabaci*. Furthermore, the nanoformulation offered better protection against the degradation and oxidation processes of natural compounds of *Lippia* under sunlight and heat conditions. In addition, the aqueous solubility was increased by a natural surfactant such as chitosan, thereby improving the insecticidal potential of this nano-emulsion and favoring its applications in the field.

However, further studies would be required to determine the thermal characterization and essential oil content of nanoparticles, the morphology and particle size of nanoparticles and the identification of the structure of compounds.

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