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Biopesticide Plants species of the mining area of Tokpli (South-Togo) effects on Okra (*Abelmoschus esculentus*) protection against *Aphtona spp*.

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ABSTRACT

Pest management is among the many challenges confronting African farmers today. In Togo, pest attack has been seen as a challenge posed on many vegetable crops producers. In pursuit to contribute to effective vegetables crops protection using local Biopesticide plants, an ethnobotanical survey conducted in the District of Tokpli to collect data from the local population identified five biopesticide plants. Among these plants, *Chromolaena odorata*, *Morinda lucida*, *Azadirachta indica*, and *Ludwigia decurrens* pure leaves extract significantly reduced the number of *Aphtona spp.* attacking Okra (*Abelmoschus esculentus)* plants in the field. Treatment Efficiency of the pure aqueous extract from mixture of leaves of these four plants, applied at the interval of 1 day and 2 days of 14 days were respectively 90% and 81% and were significantly higher than the result obtained with the chemical insecticide LAMBDA POWER applied following the recommendations of the manufacturer which was 79%.

Keywords: Biopesticide, crops protection, *Chromolaena odorata*, *Morinda lucida*, *Azadirachta indica*, *Ludwigia decurrens*.

INTRODUCTION

Many African economies are currently dependent on agriculture and mineral exploitation. In Togo, agriculture and mining play an important role in the economic balance (PARI, 2015). More than 120 industrial units are spread throughout the country and participate in economic development of the nation. Unfortunately, the economic interests related to mineral exploitation are often associated with significant negative impacts on the environment (MERF, 2010). Occupation of agricultural land by mining activities contribute to a reduction in crop production and increased food insecurities encountered in

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many African countries. Further, pest incursions, disease outbreaks due to pathogens and soils degradation do not allow farmers to produce food (Kanda et al., 2014). Pest management is the major constraint to crop production especially in garden crop production in Africa. To overcome these problems, farmers frequently use chemical fertilizers and pesticides which are very expensive and dangerous for human health and ecological systems (Hubert, 2016; MERF, 2011). Crop protection practices in gardening areas are primarily based on excessive use of synthetic pesticides which in most cases include organophosphates (27.3%) and pyrethroids (18.2%), known to be dangerous to human health and the environment (Agboyi et al., 2015). An assessment of pesticide residue contents in vegetables cultivated in urban area of Lome (capital city of Togo) has shown that 78, 57% of samples were contaminated with pesticide residues above European Union Maximum Residue Level though the level of contamination could not be considered as a serious public health problem in the studied conditions (Ahoudi et al., 2018). In developing countries, an estimated 1 to 5 million cases of pesticide poisoning occur each year, resulting in deaths of several thousands of agricultural workers including children. Although African countries import less than 10% of the world's pesticides, they account for half of the accidental poisonings and more than 75% of fatal cases; according to the World Health Organization. Moreover, each year, nearly 750,000 people contract a chronic disease such as cancer, infertility and malformation due to pesticide exposure (Tachin, 2011). Besides the health issues caused by pesticide use, more than 70 % of farmers in most African countries do not have easy access to pesticides during pest attack. The recent outbreak of the Fall Army worms on the continent which led to substantial loss in maize production not only due to the lack of knowledge regarding the FMW but also the difficulties faced by farmers in their attempt to get efficient pesticide to control the infection (Njuki et al., 2003).

In the recent years, many studies have shown the high potential of plant with fertilizer and biopesticide properties. The use of local plant species with insecticide properties that easily available is a sustainable way of producing vegetables while conserving biodiversity and protecting human health (Grzywacz et al., 2014). In Africa, there are many plants that can be used in biological control of pests.

The integration of biopesticide products from locally available plants for use in storage by the farmers in developing countries appears to be quite safe and promising (Doumma et al., 2011). *Tephrosia vogelii* Hook f. for example has been used across Africa as a pesticide and soil fertilizer (Mafongoya and Kuntashula, 2005; Neuwinger, 2004). Experiments on plant extracts of turmeric (*Curcuma longa*), lemon (*Citrus limon*), garlic (*Allium sativum),* pepper (*Capsicum frutescens*) and ginger (*Zingiber officinale*) were reported to be significantly effective against several pathogens like *Alternaria solani*, *Pythium ultimum*, *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. Lycopersici, *Staphylococcus aureus*, *Escherichia coli* and fungal strains *Candida albicans* using agar well diffusion method (Magwenya et al., 2016; Ali et al., 2017; Muthomi et al., 2017).

SCANTOGO mining companies have been exploiting limestone in Togo since 2012. The exploitation sites are based in Sikakondji located in Tokpli district which is about 95.8 km from Lome. The large population of people living in Sikakondji and its vicinity are mainly farmers who are specialized in vegetable production, such as Jute mallow (*Corchorus olitorius*), okra (*Abelmoschus esculentus*), tomato (*Solanum lycopersicum*), chili (*Capsicum annuum*), and spinach (*Solanum macrocarpon*) which are widely used in Togolese daily diet. However, exploitation of limestone in these areas considerably reduces the cultivated lands dedicated to those crops. Besides the problem caused by the limestone exploitation, the yield of those farmers is also threatened by pest attack; mainly flea beetles (*Aphtona sp*), a common pest in the area. In their attempt to reduce yield losses, the major part of the population in this mining zone use chemical pesticides for their vegetable crops protection. However, like mentioned above, those chemicals are harmful and often lead to pesticide poisoning.

The present study aimed at i) identifying plants species with biopesticide properties in farming area surrounding SCANTOGO mines SA Limestone exploitation through a prospection and an ethnobotanical survey, ii) identifying the most efficient biopesticide species in the protection of *Abelmoschus esculentus* (Okra) against *Aphtona spp*, iii) testing and identifying the best dose and application frequency the discovered species leave's extract in Okra protection against *Aphtona spp.*

MATERIALS AND METHODS

Description of the study area

The study area is mainly composed of villages bordering the SCANTOGO mining zone i.e. Sikakondji, Tokpli districts. These villages are: Ziome-Kondji, Asu-Kondji, Anagonou-Kpota, Nyinda-kondji, and Ahlimegni-kondji. Those villages are the ones in the vicinity of the limestone exploration site and their agricultural land area is occupied by more than 50% by the mining activities. This site is located in the ecofloristic area V of Togo specifically in a limestone career of SCANTOGO based in Sika-Kondji (8 Km from Tabligbo) at 06° 36'48.7" North Latitude and 1°34'44.1 " East Longitude. The region (14 km^2) is characterized by vast farmland fallow around villages, dotted with palm plantations.

Methodology of ethnobotanical surveys, floristic inventories, and biopesticide plant species selection

The surveys were carried out by interviewing individuals and groups of residents of the five villages (Ahlimegni Kondji, Anagonou Kpota, Assou Kondji, Nyinda-kondji, and Ziome kondji) bordering the exploration site and fields visit. One hundred (100) farmers were selected across the 5 villages. They were all 18 years old and above at the time of the study and the selection was done without distinction of sex, occupation, ethnicity, and level of education. The questions focused on knowledge about bioipesticide plants species, the garden vegetable crops where insecticides are most applied, and the main pathogens which attack these vegetable crops in the area. The species mentioned by people were recorded, collected and identified at the Laboratory of Botany and Plant Ecology of University of Lomé.

In order to identify some biopesticide plants species present in the study area but not mentioned by farmers in survey, two scientific reports (Mabafei, 2015; Kokou, 2016) were reviewed. An oriented floristic inventory was then obtained from the list of species identified by the survey and those identified in literature. This inventory allowed frequency determination of the different species identified. Three criteria were set in order to identify species that can be used in crop protection i.e. the species is reported in the selected literature as a plant with biopesticide properties, it must be found in the area of study and finally, it must grow naturally and spontaneously in the area of study.

Biopesticide effects of selected spontaneous plant species

To determine the biopesticide effects of the selected species, extraction of the fresh leaves was performed according to modified method of Acero (2017) and Sukhthankar et al. (2014) at the Lab of Organic Chemical and Natural Substances (Lab COSNat) of "Université de Lomé". The leaves of each species were collected and dried at room temperature in the laboratory for a week. They were then ground and sieved. Extraction was prepared for each powder sample (100 g of powder in 1 L of Alcohol). Flasks were put in the dark for 3 days and filtration was performed using Wattman#2 paper. The filtrates were used for agronomical tests on Okra (*A. esculentus*), one of the most commonly cultivated garden vegetables in the area. Besides, its production is heavily impacted by *Aphtona spp*. (the most important pathogen) in the study area. The experiment consists of 6 treatments including leaf extracts of the selected species and one control treatment. The filtrates were diluted at 10% with water (10 ml water/100 ml extract) before using. 60 mL of the obtained extract was sprayed over 100 m^2 per plot every 7 days. The evaluated parameter is the number of plant specimens from which more than half of the leaves were affected by pathogens after 9 weeks of treatment. Table 1 presents the main physicochemical characteristics of this soil in the area of study.

After evaluating the biopesticide properties of the selected plants species, an attempt to evaluate the synergistic effects of the plants species with the fourth best bio-pesticides activities was done. 100 g (25 g from each) of dried ground leaf powder of the 4 species were mixed in 4 L of local ground water (Satti et al., 2010; Udebuani et al., 2015). After stirring manually for 5 minutes, the mixture was placed in the dark for 4 days while ensuring that the solution was stirred every 24 hours, after which two successive agronomic tests were conducted to get the most economic and effective dilution dose and the efficient frequency of treatment by the extract mixture. An Experiment was carried out in a completely randomized design with three replicates per treatment. Each elementary plot (replications) size was 6 m^2. A variety of *A. esculentus* lasting 75 days was used. Seeds were sowed one week after plot preparation. After two weeks of growth, two plants were kept per pocket.

For the right concentration identification test, 25%, 50%, 75%, and 100% were the different concentrations tested under 1 L/100 m^2 (30 ml for the 3 m^2) in comparison with a chemical insecticide (LAMBDA POWER) diluted to 20% before application. The different dilutions were labelled bio-25%, bio-50% and bio 75% while the pure extract was labelled bio-100% and were applied at a frequency of 4 days as recommended for the chemical insecticide. After identifying the effective dose, a frequency test was performed to determine the most effective frequency for the treatment. The most effective dose identified from the previous test was then used at frequencies of 1 day, 2 days, 3 days and 4 days. Both tests lasted 2 weeks (14 days) after plants germination. The number of flea beetles (*Aphtona spp*., a common pathogenic insect in the study area) per plot is the main parameter collected daily for the 2 tests.

Data analysis

Simple analysis of variance (ANOVA) was used to analyze the data using SPSS 22.0 (IBM, 2013). Significant differences between means were compared using Fisher's least-significant difference (LSD) test at a probability level of 95% ($P \leq 0.05$). Treatment efficiency was calculated using the following formula:

TE= ((NCT – NOT)/NCT) x 100 (%)

TE: Treatment Efficiency; NCT: Number of *Aphtona spp*. on control treatment (Treatment without insecticide); NOT: Number of *Aphtona spp*. on other treatment.

Tableau 1: Physico-chemical properties of the soil in the area of study.

Organic matter	pH	Nitrogen (%d.m.)	Potassium (ppm)	Phosphorus (ppm)	Clay (%)	Lime (%)	Fine sand (%)	Coarse sand (%)	gravel (%)
5.07	7.63	0.0134	1252.75	2.15	4.5	9.5	58.2	27.4	0,3

RESULTS

Ethnobotanical Surveys

Survey results (Table 2) shows that women are more involved in vegetable production than men (60% of women against 40% of men). Relative to the ethnobotanical survey, 60 women and 40 men were surveyed (Table 2). Findings show that less than half of the population has knowledge on biopesticide plants (43%) and only 34% utilize these biopesticide plants for crop protection (Figures 1a and 1b). Compared to the modes of use, extraction and infusion are the most practiced methods, 65 and 27.5% respectively (Figure 2a). Regarding population perception on biopesticide efficacy, more than 40% recognize a high efficiency of bio-pesticides while 12% said bio-pesticides are not effective (Figures 2b).

Biopesticide species and important vegetables crops of the study area

Ethnobotanical surveys, floristic inventories and bibliographic research have identified five (5) biopesticide plants species in the area (Figure 3). They are *Chromolaena odorata*, *Mallotus oppositifolius*, *Morinda lucida*, *Azadirachta indica*, and *Ludwigia decurrens* with respective relative frequencies of 12.5, 9.37, 6.25, 6.26 and 1.56. All these species have met the criteria for their selection as biopesticide.

Ethnobotanical surveys have identified 14 vegetable crops in the study area including the vegetable garden coret (*Corchorus olitorius*), okra (*Abelmoschus esculentus*), tomato (*Solanum lycopersicum*), chili (*Capsicum annuum*), and spinach (*Solanum macrocarpon*) as the most popular. The main pathogens against vegetable crops in the study area are insects (80%) and worms (20%). Of these insects *Aphtona spp.* and *Harmonia axyridis* are the most notable.

Effects of bioinsecticid plants extracts against *Aphtona* spp.

The results of the first test show that *Ludwigia decurrens*, *Morinda lucida*, *Chromolaena odorata* and *Azadirachta indica* are the 4 best biopesticide species (Figure 4). Difference between the treatments is statistically significant at the probability threshold of 5%. There were no differences observed between the treatment of Okra plants by the specy *Mallotus oppositifolius* and the control treatment. *Azadirachta indica, Chromoloena adorata and Morinda lucida* were not significantly different in their effects on Okra protection against the flea bettle although they performed significantly better than *Mallotus oppositifolius.* According to the efficacy test, the most effective plant is *Ludwiga decurrens* which perform significantly better than the other four (4).

Effects of the 4 best biopesticide plant extracts mixture on Aphtona spp. compared to a common chemical insecticide LAMBDA POWER

To determine the effects of the bio-pesticide under study, 100 g of mixed leaves powder (25 g leaves powder from each of the 4 plants species with the strongest bio-pesticide activity on Aphtona spp) was diluted in 4 L of local drilling water to test the bioinsecticide activity of the combination of *Azadirachta indica, Chromoloena adorata, Morinda lucida* and *Ludwiga decurrens* leaves extract. The obtained extract was then diluted and test at different concentrations to see their effects in the control of Okra attack by *Aphtona spp*. All the treatments had significantly different effects on the number of flea beetles and the different dilutions have proportional effects to their concentration. For example, the pure extract (bio-100%) performed significantly better in reducing the number of flea beetles compared to the 25%, 50% and 75% dilution and the 75% dilution effects were significantly better compared to the 50% dilution. However, the number flea beetles obtained in the case of 25% dilution treatment was similar to the number observed for control treatment. Another fact is that the treatment of the okra plants by the chemical insecticide had better results compared to the different dilution of the obtained biopesticide (Figure 5a).

For the best application frequency, the pure extract (bio-100) used at the frequency of 1 day was proved efficient in controlling effectively the number of *Aphtona spp* per plot compared to the other frequencies of application (2 days, 3 days and 4 days) (Figure 5b). Besides, the treatment efficiency of the different frequency of application of the pure extract was calculated and compared to the efficiency of the treatment by the chemical insecticide used at the frequency of 4 days as recommended by the manufacturer (Figure 6). The results have shown that the treatment by the pure bio-pesticide extract at the frequency of 1 day and 2 days was respectively more effective compared to the application of the chemical insecticide (TE of 90% and almost 81% for the treatment by the pure extract at a frequency of 1days and 2 days respectively compared to a TE of 79% for chemical insecticide). The treatment efficiency by the biopesticide at the frequency of 3 and 4 days were not significantly different from the result obtained with the chemical insecticide.

Tableau 2: Number of respondents per village and gender during ethnobotanical surveys.

Villages	Women	Men	Total
Ahlimegni-Kondji	10	7	17
Anagonou-Kpota	9	8	17
Assou-Kondji	25	8	33
Nyinda-kondji	8	9	17
Ziome-kondji	8	9	17
Total	60	40	100

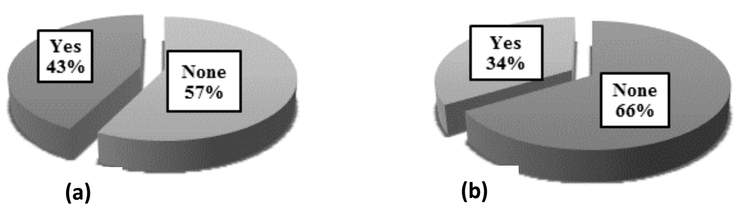


Figure 1: (a) Knowledge and (b) use of bio-pesticide plants by the population.

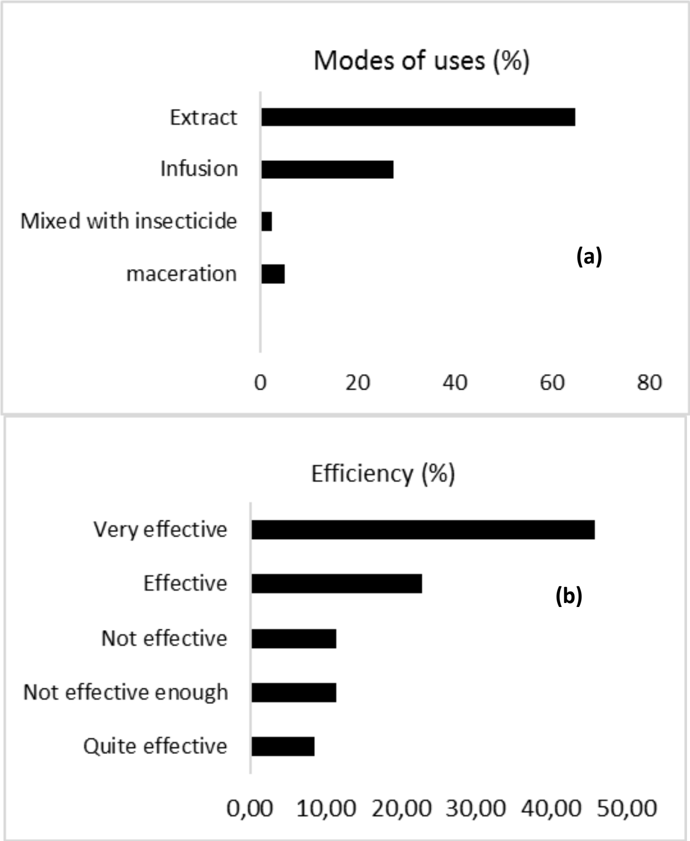


Figure 2: (a) Modes of use and (b) populations perception on bio-pesticide efficiency.

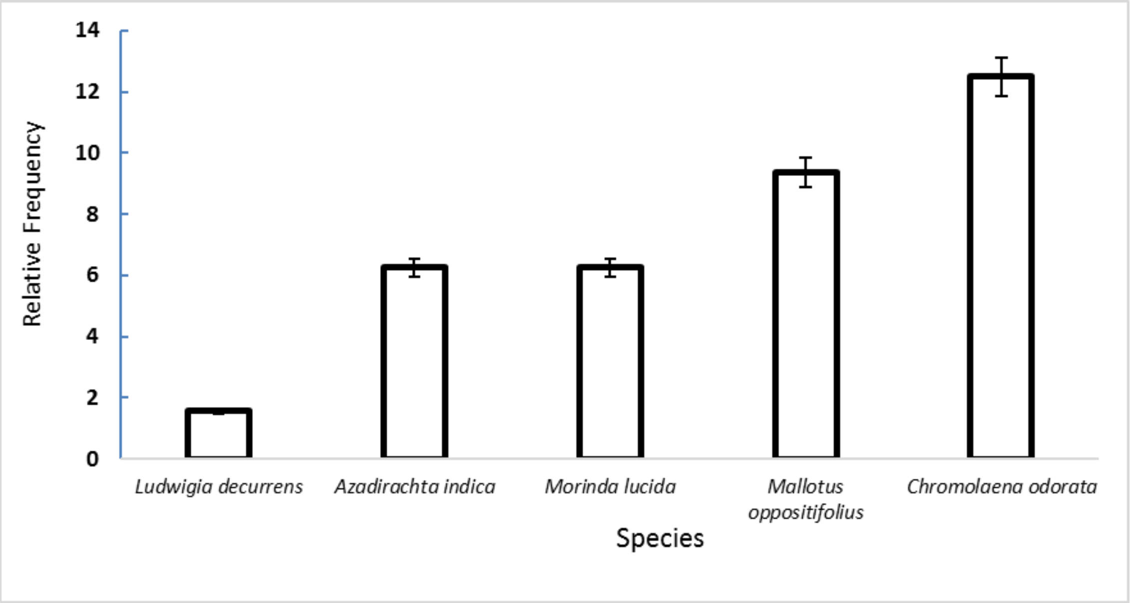


Figure 3: Natural bio-pesticide plant species identified in the study area.

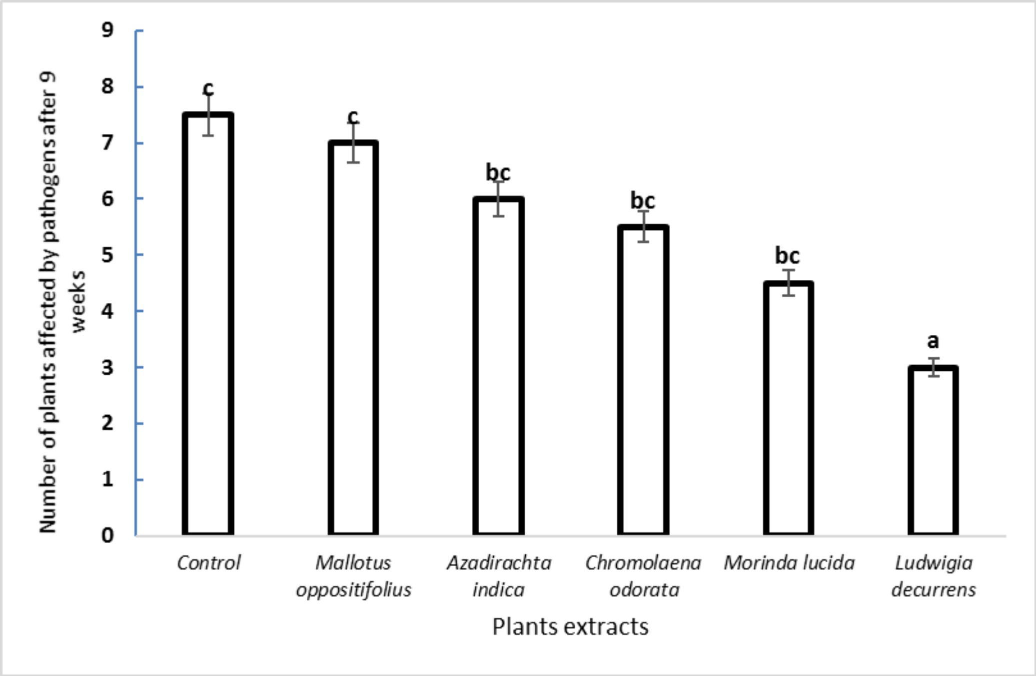
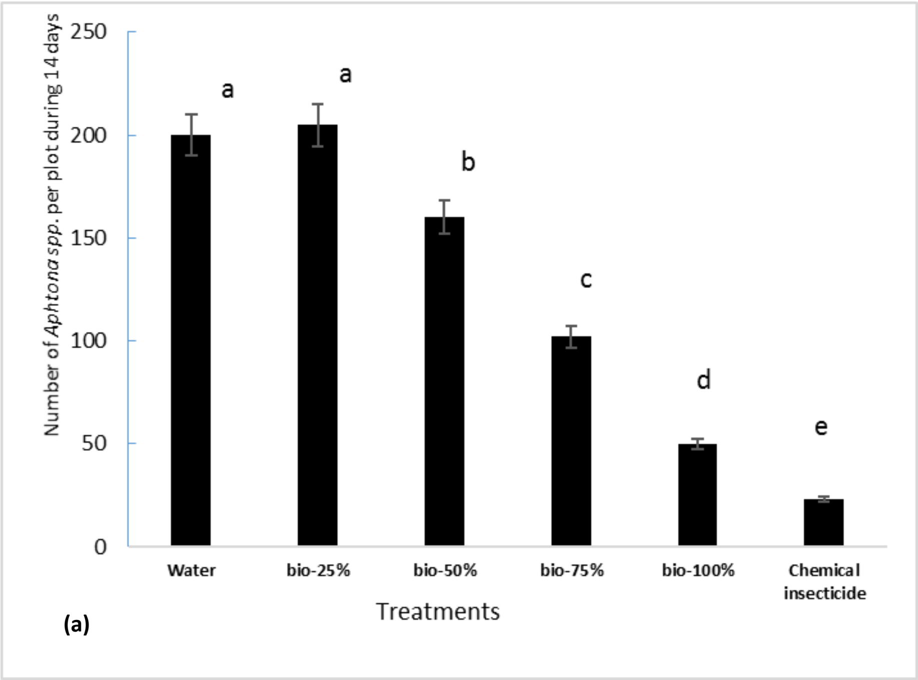


Figure 4: Effects of bio-pesticide species on the number of plants affected by pathogens after 9 weeks of treatment.



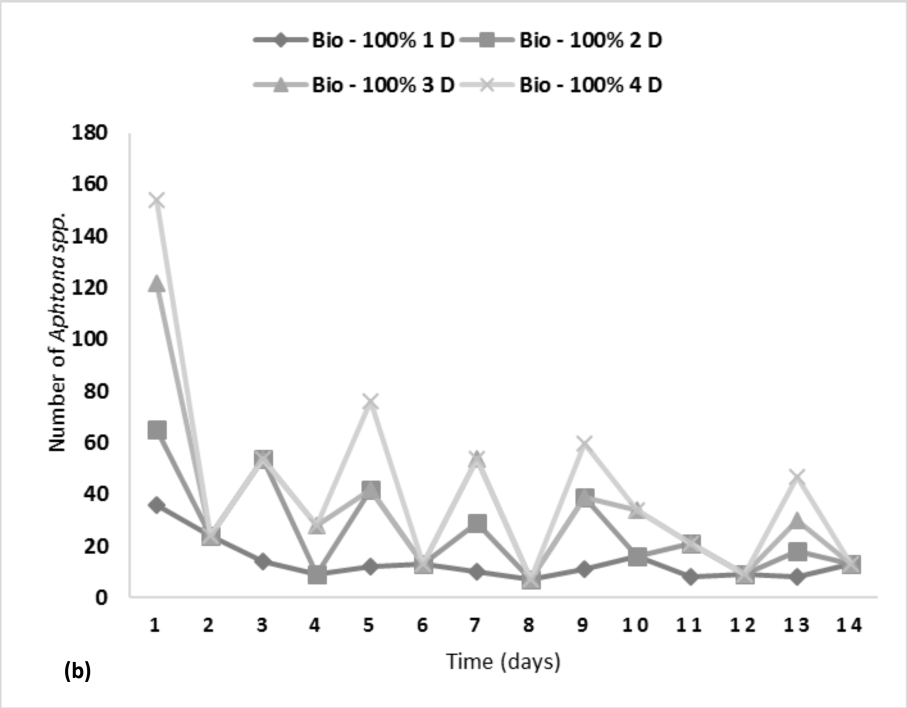


Figure 5: (a) Effects of bio-pesticide doses on the number of *Aphtona* ***spp*** (Water= Control treatment; bio-25%= mixture diluted at 25%; bio-50%= mixture diluted at 50%; bio-75%= mixture diluted at 75%; bio-100%= mixture without dilution). **(b)** Effects of the pure mixture extract applied at different frequencies on the number of *Aphtona spp.*

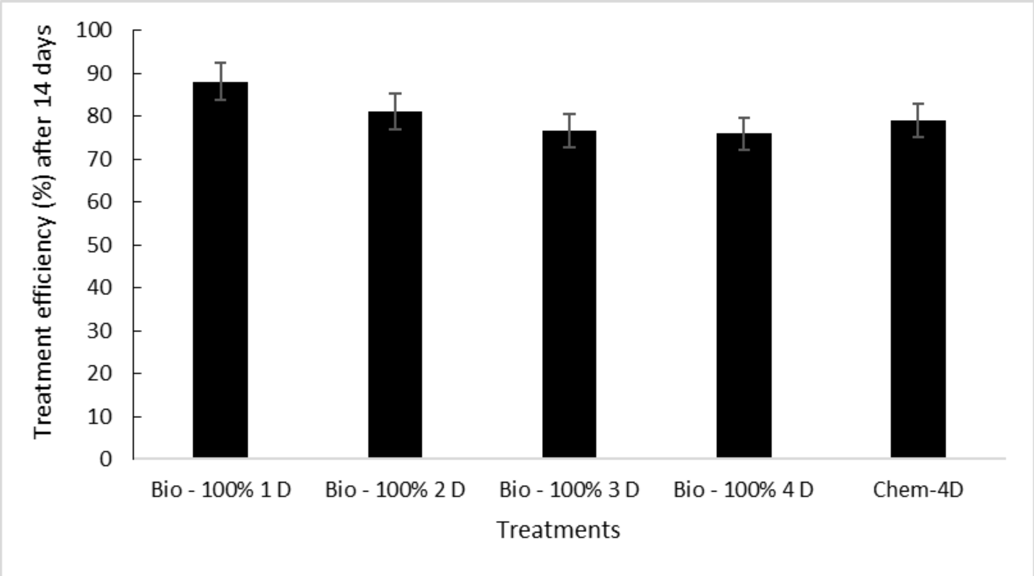


Figure 6: Treatment efficiency (a) (Bio-100% 1D= Pure mixture applied at a frequency of 1 day; Bio-100% 2D= Pure mixture applied with at a frequency of 2 days; Bio-100% 3D= Pure mixture applied with at a frequency of 3 days; Bio-100% 4D= Pure mixture applied with at a frequency of 4 days; Chem-4D= Chemical insecticide applied at a frequency of 4 days).

DISCUSSION

Results of ethno-botanical surveys show that women are more involved in this vegetable production. These results are in discordance with those of Mondedji et al. (2015) who showed that men are more involved in gardening in South-Togo (72% against 28% for women). Indeed, in Yoto District, women are more involved in agriculture than men. According to a recent report by Heidelbergcement Foundation, there is a strong involvement of women in agricultural groups, i.e. 77% against 23% of men in the study area (HEIDELBERGCEMENT, 2017).

In the study area, 43% of the participants to our survey have knowledge of bio-insecticide plants and only 34% of the population uses plant extracts to protect crops against pests. This result is in agreement with those of Mondedji et al. (2015) who showed that chemical control is the main method of pest control in South-Togo and 47% of the population use bio-pesticides. The percentage of biopesticide users in the study area is lower than 47%. This suggest that chemical insecticides are heavily used for vegetable production. These chemical insecticides could be responsible of several diseases observed in the population in Togo. For example, symptoms of severe intoxication have sometimes been observed (breathing difficulties 6.7% and convulsion 1.2%) among users of chemical insecticides in Togo (MERF, 2011).

Aphtona spp. is the most important pest in the study area. The negative impacts of these insects on vegetable crops have already been reported by some authors. Lambert (2010), Bidiga and Nacro (2016) have pointed out that these insects heavily impact the yield of crops by destroying the plant's leaves. The Control of these pests appears important to improve vegetable production in the area of study.

Morinda lucida, Chromolaena odorata, Azadirachta indica and Ludwigia decurrens are the best bioinsecticide plants of the study area. *Morinda lucida*, *Chromolaena odorata* and *Azadirachta indica* have already been cited in literature as being plants that can be used in crop protection against pests. Studies on the aqueous and methanolic extracts of *Morinda lucida* and *Chromolaena odorata* have shown that these plants contain aromatic organic compounds capable to repel insects. Ajayi et al. (2012) and Owolabi et al. (2014) have studied the bio-insecticide properties of *Morinda lucida* leaves and seeds. Other studies on *Chromolaena odorata* have shown that its extracts can control female anophelines (Sukhthankar et al., 2014; Udebuani et al., 2015; Acero, 2017). The Works on Neem (*Azadirachta indica*) are well known in the world. Indeed, its bio-insecticide potential is explained by the presence in leaves and seeds of active ingredients capable of repelling or killing insect pest at high doses (Lokanadhan et al., 2012; Mondal and Chakraborty, 2016). The work of Pathak and Tiwari (2012) showed that the acetone extract of neem seeds is effective against larvae of *Corcyra cephalonica* Staint., a pest of rice. The aqueous extract of neem leaves is effective in controlling cowpea pathogens (Baidoo et al., 2012) and the hydromethanolic extract of neem leaves has significantly reduced population of *Plutella xylostella* (Lepidoptera : Plutellidae) and *Lipaphis erysimi* (Hemiptera : Aphididae) and increased the yield of treated cabbage compared to the control in vegetable crop production area of .Kotokoukondji in Lomé (Mondédji et al., 2017).

Among the species identified in our study, only *Ludwigia decurrens* is less studied in literature but showed the best results in protecting okra against *Aphtona spp*. The most important study on this species was on the phytochemical screening properties of it crude leaf extracts which revealed the presence of alkaloids and tannins (Oyedeji et al., 2011). Another study has shown that L. *decurrens* extracts induced mortality rate of the 15 day old seedlings of *C. olitorious* (control: 5.00%, *L. decurrens*: 17.50%) and a significant decrease in seedling elongation (Sakpere et al., 2010). Advanced studies should to be conducted on this species in order to better know the active compounds responsible for its bio-insecticide activity.

The extract obtained from the mixture of leaves of the four species was tested at different concentrations in the control of Okra infection by *Aphtona spp*. The results obtained showed that the pure extract significantly reduced the number of flea beetles on Okra plants. However, the chemical insecticide LAMBDA POWER performed better than the pure extract when both were applied at a frequency of 4 days. Furthermore, the pure extracts of leaves mixture used at a 4 day treatment frequency is efficient in controlling pathogenic insects (*Aphtona spp*.) at a 76% efficiency rate compared to the chemical insecticide LAMDER POWER (79%). This fact can be explained by the low afterglow of bio-pesticides compared to chemical insecticide (Deravel et al., 2014). Therefore, when applied at the frequency used for a chemical insecticide which has a high afterglow, the efficiency of a biopesticide is reduced compared to the chemical insecticide. The application of the pure extract at the frequency of 1 and 2 days have a higher treatment efficiency compared to the efficiency of the recommended frequency application of the chemical. This result is very interesting and is in favor of the use of bio-insecticides against pests in market gardening system in the study area. In fact, forty-seven plant species have been listed for their toxicity to different insect species and their reactions against pathogens as synthetic insecticide (Talukder, 2006). For example, neem (*Azadirachta indica)* extracts contain about 100 biomolecules among which triterpenes (limonoids) are the most important, causing 90% of the effects on most of the insect pests. It has already been reported that neem extracts have useful insecticidal properties against many lepidopteran insect pests, larval and nymphal (Campos et al., 2016; Ali et al., 2017) . The main compounds of the essential oil of *M. lucida* were the oxygenated monoterpenoids, 1.8-cineole (43.4%), and α-terpinyl acetate (14.5%), and the monoterpene hydrocarbons, mostly sabinene (8.2%) and β-pinene (4.0%) (Owolabi et al., 2014). These compounds have a high insecticidal properties according to Hennia et al. (2019). The associated effect of these substances from different plant species could be responsible for the efficiency of the extracts mixture.

Conclusion

The objective of this study was to assess the use of natural local bio-insecticide plant species in vegetable crop protection in the district of Tokpli. Four natural bio-insecticide plant species were identified and tested. Three of them are well known for their bio-insecticide activities while *L. decurrens* was less studied. Further studies must be conducted on this plant to characterize and isolate the active compounds responsible for this biopesticide activity. Furthermore, the study has shown that the effects of leaf extract mixture from the 4 species has interesting treatment efficiency at the application frequency of 1, 2 and 4 days and can be successfully used in vegetable crop protection against pests.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR'S CONTRIBUTIONS

Agronomical tests and field data collection were done by TO. The investigation of the efficient concentration of the biopesticide, literature review and the statistical analyses were done by PKK. Aqueous extract preparation was done by SO. Species identification was done by AM. WI performed the physico-chemical characterization of the soil. The supervision of the whole work was done by TK. All the authors read and approved the final manuscript.

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