Effectiveness of plants extracts and mycoinsecticide on management of cowpea flower thrips *Megalurothrips sjostedti* (Thysanoptera: Thripidea) under field conditions

ABSTRACT

To promote the use of natural substances for better management of the cowpea thrips *Megalurothrips sjostedti*, the potential of aqueous extract of *Azadirachta indica*, *Boswellia dalzielii* and *Metarhizium anisopliae*, alone and their combinations in comparison with the synthetic chemical insecticide (Decis) were tested. The experiments were carried out in two cowpea varieties under field conditions within the Sudano-Sahelian agro-ecological zone of Cameroon. Trials were arranged in a completely randomized block design with nine treatments. The nine treatments consisted of a negative control, *A. indica*; *B. dalzielii*; *M. anisopliae*, *A. indica*+*B. dalzielii*; *M. anisopliae*+*B. dalzielii*; *M. anisopliae*+*A. indica*; *M. anisopliae*+*A. indica*+*B. dalzielii* and Decis. Each treatment replicated four times. *Vigna unguiculata* plants were sprayed from flowering stage thrice with insecticidal products at 5 day intervals. The density of adults and larvae thrips was assessed on 5 flowers per block during 5 days after the last spraying of treatments. Results showed that, there were more thrips on Bafia variety than B125 variety ($p<0.0001$). All the tested treatment significantly ($p<0.0001$) reduced the cowpea flower thrips of 30% for *A. indica*, *B. dalzielii* and *A. indica*+*B. dalzielii*, 75% for *M. anisopliae* and its various combinations, and of 90% for Decis. Plants extracts, mycoinsecticide and their combinations considerably reduced damage induced by thrips compared to the negative control. *A. indica*, *B. dalzielii*, *M. anisopliae* and their combinations showed ability as natural traitement for the management of thrips in *V. unguiculata*. These products do not only protect *V. unguiculata* crop from its major pest, but also preserve the environment from harmful effects induced by the use of synthetic commercial pesticides.

Keywords: *Azadirachta indica*; *Boswellia dalzielii*; *Metarhizium anisopliae*; *Vigna unguiculata*; Efficacy.

1. INTRODUCTION

Agriculture plays an important role in the development of many countries (Adeoti *et al*. 2002). Hence, the cultivation of legume crops such as cowpea is necessary to promote diversified incomes. In Cameroon, cowpea is the staple food in the entire country. It is used in several dishes (Nielsen *et al.* 1997) by all the ethnics in the country and it is called in some localities 'meat of poor', because it sometimes replaces animal protein in some dishes. Cowpea is cultivated in almost all the different agroecological zones of the country due to its economic value. It generates incomes for the farmers and provide them with food during hungry gap (Odion *et al*. 2007). In addition to its nutritional qualities (Ndiaye 1996), cowpea improves the soil fertility by its ability to naturally fix the atmospheric nitrogen (Okigbo *et al*. 1978). However, very useful, the yield of this crop is slow in Cameroon even though the demand in cowpea grain is still growing.

Unfortunately, cowpea cultivation is faced with several constraints, such as fungal, bacterial and viral diseases (Singh *et al.* 1997), and insect pests, all of which are responsible for serious damages yield and losses (Tamò *et al.* 1993). Among the insect pests, cowpea flower thrips (*Megalurothrips sjostedti* Trybom) has been reported as the most harmful, because more than 80% of yield loss is attributed to this pest (Omo-Ikerodah *et al*. 2009). This justifies the necessity to protect cowpea from this damaging insect pest.

Previous studies have shown that, management of cowpea thrips is attributed to the use of synthetic insecticides (Alghali 1992; Karungi *et al.* 2000). Deplorably, the use of these synthetic pesticides has many harmful effects on other organisms and the environment (Bambara and Tiemtoré 2008). Commercial insecticides are expensive and also acidify the soil in case of misuse, while their residues are toxic to non-target organisms, in addition to resistance development by insect pests (Immaraju *et al.* 1992; Margni *et al.* 2002). More so, they pollute surface and groundwater through leaching (Ouédraogo 2004). Therefore, the promotion of eco-friendly control strategies to manage the density of *M. sjostedti* in field is important.

Several plant extracts provide natural insecticides, and can be used as substitutes to synthetic chemical insecticides (Barry *et al*. 2017). Neem tree (*Azadirachta indica* A. Juss) has been shown as a potential insecticidal plant (Mouffok *et al*. 2008). Its extract has been shown efficacy in controlling many harmful insect species (Harouna *et al*. 2019). Other plants such as *Boswellia dalzielii* Hutch has been used to protect stored food grains (Kémeuzé *et al*. 2012). These properties make these two plants potential alternatives to synthetic pesticides. The use of enthomopathogenic fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin against the flower thrips was discussed by Mfuti *et al*. (2021), and has shown insecticidal potential against cowpea flower thrips (Ngakou *et al.* 2008).

Investigating the potential of *B. dalzielii*, *A. indica* and *M. anisopliae* to management of cowpea flower thrips was carried out in this study

2. MATERIALS AND METHODS

2.1. Study area

The experiment was carried out in Béguélé-Maroua located in the subdivision of Maroua, Far-North region of Cameroon. The climate of the region is Sudano-Sahelian, characterized by a mean annual rainfall of about 757.2 mm and a mean annual temperature of about 28℃. This region has two seasons, a dry season from

November to May and a raining season from June to October. The vegetation in this area is characterized by shrub savannah locally arboreous with various grasses. Trials were conducted for two consecutive years (2014 and 2015), and the field GPS coordinates are latitude 10°35'58,3" N; longitude 14°11'28,4" E; altitude 450±2 m.

2.2. Plant materials

Plant materials consisted of two cowpea varieties: the local Bafia multiplied locally during subsequent work, and the B125 provided by the 'Institut de la Recherche Agricole pour le Développement (IRAD)' Maroua. The B125 variety was an early maturing variety (75 days), whereas the Bafia variety was an intermediate maturing variety (85 to 95 days). The leaves of *A. indica* (Meliaceae) and *B. dalzielii* (Burseraceae) were collected in Maroua at the locations of geographical coordinates: latitude 10°35'27,1" N; longitude 14°17'32,34" E; altitude 409±2 m above sea level, and latitude 10°37'37,95" N; longitude 14°12'4,19" E ; altitude 457±2 m above sea level respectively.

2.3. Cropping calendar

The sowing was carried out on August 23rd for the first season (2014), and August 24th for the second (2015). The cowpea crop reached their maturity after 75 and 95 days after sowing respectively for B15 and Bafia varieties concerning the first year of cropping (2014). While in the second year, the same varieties in the same period reached their maturity after 75 and 87 days of sowing respectively.

2.4. Formulation of Insecticides Products

The aqueous extract of *A. indica* leaves was obtained in accordance the method recommended by Sahel People Service. Five litres of solution was obtained by macerating 1 kg of *A. indica* fresh leaves in water. The resulting concentrated macerate was then diluted to 10% with water and filtered through a 0.4 mm mesh tissue, for a working concentration of 20 g/L. The same method was applied to obtain the aqueous extract of *B. dalzielii*. The *M. anisopliae* based solution was obtained using the method described by Ngakou *et al*. (2008), which requires the mixture of 50 g of *M. anisopliae*, 700 mL of kerosene and 300 mL of cotton oil (Diamaor stamp). *Metarhizium anisopliae* was prepared at a concentration of 10 g/L. The myco-insecticide *M. anisopliae* originated from IITA Cotonou-Benin, while Deltamethrin-based synthetic insecticide (Decis) was purchased from a phytosanitary store and was prepared by diluting 3 mL of Decis in 15 L of water.

2.5. Experimental layout and Treatments

Plants were grown on flat surface measuring 57.75×25 m^2. The experimental field was divided into two parts representing each a cowpea variety separated by 4 m path. The experimental plots representing the treatments were 4.5×1.5 m^2 for B125 variety, and 4.5×2.25 m^2 for Bafia variety. Seeds were planted at 50 cm distance from one plant to another, and the distance between rows of plants was also 50 cm for the early maturing variety. On the intermediate maturing variety, the distance between plants was 50 cm but between rows its was 75 cm; giving a population of 40 plants per plot for each variety. Treatments were sprayed using four distinct manual gauge sprayers (AgroPro stamp) purchased from a phytosanitary store, each corresponding to a specific insecticidal product. For multi-product treatments, each component was sprayed separately. Treatments were applied early in the morning between 6 and 8 a.m, 3 times at 5 days interval, as soon as the appearance of the first flower was noticed. The experimental design applied for each variety was completely randomized which consisted of 9 treatments, each replicated 4 times.

The treatments were: T1, negative control representing plots that did not receive any treatment; T2, plots treated with aqueous *A. indica* leaves extract; T3, plots treated with aqueous *B. Dalzielii* leaves extract; T4, plots treated with *M. anisopliae* formulation; T5, plots treated with the combination of *M. anisopliae+A. indica*; T6, plots treated with the combination *M. anisopliae+B. dalzielii*; T7 plots treated with the combination *A. indica+B. dalzielii*; T8, plots treated with the combination of the three bioinsecticides *M. anisopliae+A. indica+B. dalzielii*; T9, plots treated with the synthetic insecticide Decis.

2.6. Assessed Parameters

The assessed parameters were thrips density and leaf damage. All these parameters were assessed compared to the control. The evaluation of adults and larvae of thrips were carried out on 5 flowers per block during 5 days after the three spraying (Nderitu *et al*. 2007; Ngakou *et al*. 2008) for all treatments. The leaf damages were determined by observing and counting the number of brown desiccated marks or holes on perforated cowpea leaf for each treatment.

2.7. Statistical Analysis

The statistical analysis was carried out using the SAS software version 9.1. The number of adults and larvae, and cowpea leaf damage were subjected to the analysis of variance (ANOVA) to split the means between all the treatments. The Student-Newman-Keuls test at $p<0.05$ was used to compare the different treatments and the T-test to compare two varieties in the same year and or the same variety the two years for cropping concerning the different assessed parameters (adults and larvae thrips populations, and leaf damage).

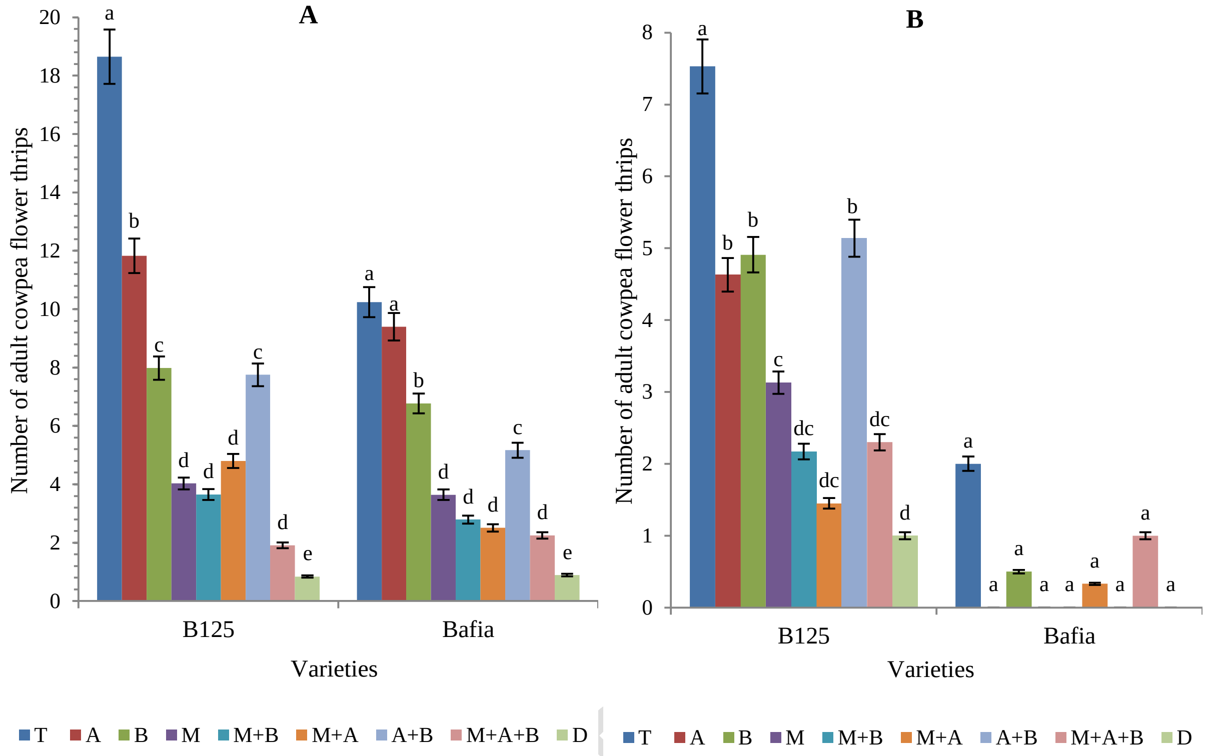
3. RESULTS

3.1. Impact botanicals application on the thrips population density

3.1.1. Adults

There were more adult thrips on the cowpea flowers of B125 variety (6.82±0.23) than Bafia variety (5.00±0.19) in 2014 (Df=1302.5; t=6.04; $p<0.0001$) and 2015 (B125=3.59±0.18; Bafia=0.56±0.22; Df=42.33; t=10.43; $p<0.0001$). All treatments applied to cowpea variety B125 significantly ($p<0.0001$) reduced the density of adult thrips population compared to the negative control during the 2014 cropping season (Figure 1A). Among the natural insecticidal treatments, *A. indica* extract with 30% adult thrips reduction was the least effective treatment. *Metarhizium anisopliae* and its various combinations reduced the density of adult thrips by 75%, but the synthetic insecticide Decis was more effective in reducing the density of adult thrips by 90%. On Bafia variety, apart from treatment *A. indica* which had many adult thrips as in the negative control, the other treatments significantly ($p<0.0001$) reduced the adult thrips population density compared to the negative control. *Boswellia dalzielii* extract was only able to reduce 20% of adult thrips. Despite being more effective than plant insecticides, *M. anisopliae* and its various combinations (*M. anisopliae* + *A. indica*, *M. anisopliae* + *B. dalzielii*, and *M. anisopliae* + *A. indica* + *B. dalzielii*) were less effective than Decis.

In 2015 (Figure 1B.), treated cowpea plants with different insecticidal formulations significantly reduced the density of adult thrips compared to the negative control ($p<0.0001$) on variety B125. Treatments of *A. indica* and *B. dalzielii* reduced the density of adult thrips by 30%, and appeared less than the other insecticidal treatments. As in 2014, *M. anisopliae* combinations were the most effective natural insecticides but did not reach the efficacy of Decis. There was no significant difference between the different insecticidal treatments and the negative control on the Bafia variety in 2015 (p=0.480).



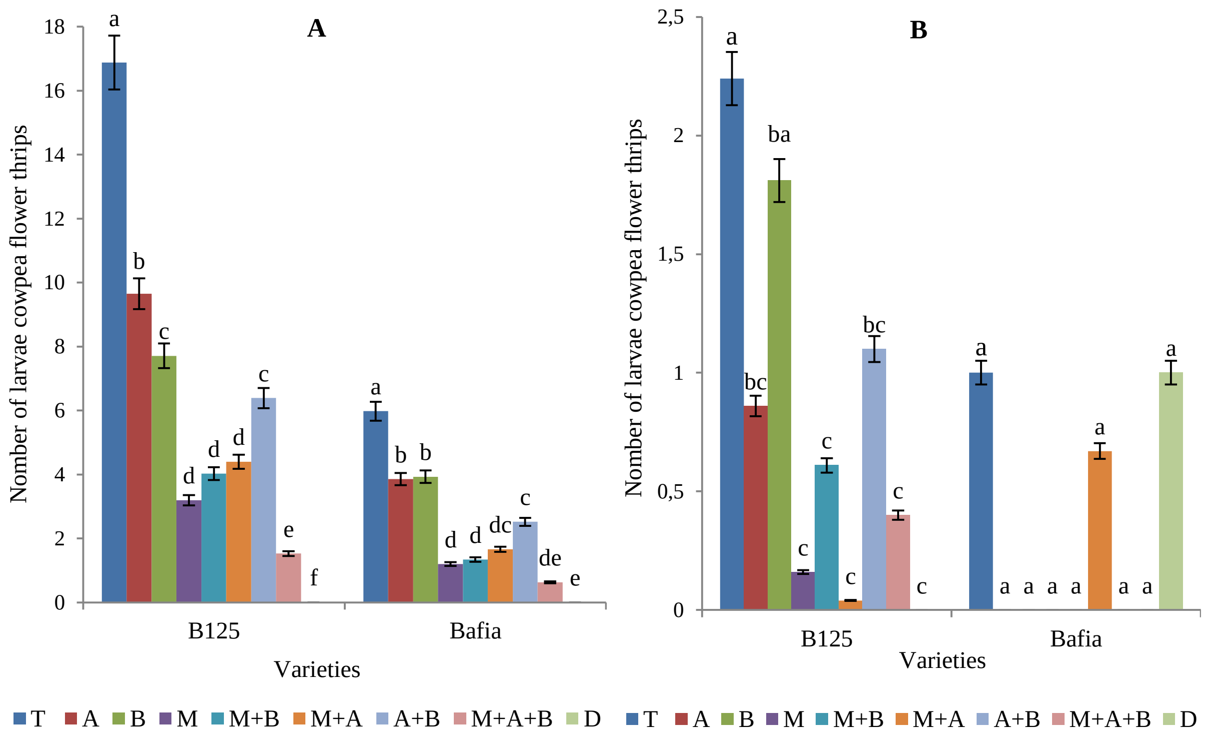
T: Negative control; A: *A. indica*; B: *B. dalzielii*; M: *M. anisopliae*; M+B: *M. anisopliae+B. dalzielii*; M+A: *M. anisopliae+A. indica*; A+B: *A. indica+B. dalzielii*; M+A+B: *M. anisopliae+A. indica+B. dalzielii*; D: Decis. For each cowpea variety bars denoted by the same upper-case letter are not significantly different between treatments at the indicated level of probability (p<0.05) (Student–Newman–Keuls test).

Fig 1. Variation of adult thrips population density between treatments of the cowpea B125/Bafia varieties in 2014 (A) and 2015 (B).

3.1.2. Larvae

The effect of natural insecticide on the thrips larvae population was similar to that on adults. There were more thrips larvae on cowpea B125 variety (5.97±0.22) than Bafia variety (2.43±0.12) in 2014 (Df=1125.6; t=13.65; p<0.0001), and in 2015 (B125=0.80±0.10; Bafia=0.31±0.17; (Df=26.48; t=2.44; p<0.0215). It was evident that all insecticidal formulations significantly (p<0.0001) reduced the larvae thrips population compared to the negative control of both cowpea B125 and Bafia varieties in 2014 (Figure 2A). The synthetic insecticide Decis eliminated all of the larvae thrips on flowers of the two varieties. On variety B125, despite a 50% reduction in larvae, *A. indica* extract was the least effective natural insecticide. The ternary combination, *M. anisopliae+A. indica+B. dalzielii* was the most effective natural insecticide with 90% reduction of larvae on both B125 and Bafia varieties. *Azadirachta indica* and *B. dalzielii* were the least effective natural insecticide treatments on Bafia variety with 30% reduction.

In 2015, all natural insecticide applied to variety B125 significantly reduced the larvae thrips population density compared to the negative control (p<0.0001), that was not the case on variety Bafia (p=0.5395), where the different formulations had the same performance (Figure 2B). On variety B125, *B. dalzielii* extract was the least effective insecticidal treatment with a 25% reduction in larvae. The effect of *M. anisopliae* treatment and synthetic insecticide Decis was similar in term of larvae reduction.



T: Negative control; A: *A. indica*; B: *B. dalzielii*; M: *M. anisopliae*; M+B: *M. anisopliae+B. dalzielii*; M+A: *M. anisopliae+A. indica*; A+B: *A. indica+B. dalzielii*; M+A+B: *M. anisopliae+A. indica+B. dalzielii*; D: Decis. For each cowpea variety bars denoted by the same upper-case letter are not significantly different between treatments at the indicated level of probability ($p<0.05$) (Student–Newman–Keuls test).

Fig 2. Variation of larvae thrips population density between treatments of the cowpea B125/Bafia varieties in 2014 (A) and 2015 (B).

3.2. Influence of natural treatments on leaf damage

The results obtained after the application of the treatments on the two cowpea varieties in the 2014 and 2015 cropping seasons are shown in tables 1 and 2. In general, there was more damage on B125 variety (3.86±0.20) leaves' than those on Bafia variety (3.34±0.14) in 2014 ($t=2.11$; $p=0.0357$). The contrary tendency was observed in 2015 (B125=3.49±0.13; Bafia=12.06±0.69; $t=12.07$; $p<0.0001$). It appears that all the insecticidal treatments significantly reduced the damage on cowpea leaves compared to the negative control in 2014 and 2015 on both varieties and reduction significantly varied according to insecticidal preparations (B125; $p<0.0001$ in 2014 and 2015; Bafia: $p<0.0001$ in 2014 and 2015).

In 2014, the natural insecticides applied to variety B125 equally protected cowpea leaves as Decis (Table 1), except *A. indica* and *B. dalzielii* extracts, which were least effective than the Decis. On the Bafia variety, *A. indica* treatment was the least effective natural insecticides. *Metarhizium anisopliae* and the combined treatments protected the leaves better than *B. dalzielii* extract alone, which was more effective than *A. indica* extract. Overall, Decis was the most effective treatment.

Table 1. Differences in the mean holes number on cowpea leaves as affected by treatments in 2014.

Treatments	**Cowpea varieties in 2014**		
	B125	**Bafia**	**t values**
Control	9.75 ± 0.77^a	8.40 ± 0.48^a	3.64**
A. indica	6.15 ± 0.68^b	4.65 ± 0.32^b	4.88**

B. dalzielii	4.95 ± 0.53^{b}	3.75 ± 0.23^{c}	5.08**
M. anisopliae	2.60 ± 0.25^{c}	2.44 ± 0.26^{d}	1.08
M+B	2.50 ± 0.21^{c}	2.25 ± 0.21^{d}	2.06
M+A	1.73 ± 0.20^{c}	2.70 ± 0.22^{d}	7.99***
A+B	2.95 ± 0.29^{c}	2.80 ± 0.25^{d}	0.95
M+A+B	2.00 ± 0.21^{c}	2.10 ± 0.14^{d}	0.97
Decis	1.30 ± 0.11^{c}	0.90 ± 0.15^{e}	5.26***
F	39.04***	65.97***	
Means (Df=605.82)	3.86±0.20	3.34±0.14	2.11*

C: Negative control; A: *A. indica*; B: *B. dalzielii*; M: *M. anisopliae*; M+B: *M. anisopliae + B. dalzielii*; M+A: *M. anisopliae + A. indica*; A+B: *A. indica + B. dalzielii*; M+A+B: *M. Anisopliae + A. indica + B. dalzielii*; D: Decis; ns: p>0.05; *: p<0.05; **: p<0.001; ***: p<0.0001. For each cowpea variety values of the same column denoted by the same upper-case letter are not significantly different between treatments at the indicated level of probability (Student–Newman–Keuls test).

In 2015, natural insecticides protected cowpea leaves of the B125 variety as Decis did (Table 2). *Azadirachta indica* treatment was even least effective, while the ternary combination *M. anisopliae+A. indica+B. dalzielii*, and the binary combination *M. anisopliae+A. indica* were more effective than Decis (synthetic insecticide). On Bafia variety, all *M. anisopliae* treatments recorded the same performance as Decis in cowpea leaf protection, whereas treatments of *A. indica*, *B. dalzielii* and their combinations were the least effective natural insecticides. However, they significantly suppressed damage induced on leaves of both cowpea varieties in the two experimental years compared to the control.

Table 2. Differences in the mean holes number on cowpea leaves as affected by treatments in 2015.

Treatments	**Cowpea varieties in 2015**		
	B125	**Bafia**	**t values**
Control	7.68 ± 0.60^{a}	28.08 ± 3.53^{a}	13.95***
A. indica	4.43 ± 0,36^{b}	18.28 ± 2.13^{b}	15.70***
B. dalzielii	3.28 ± 0.30^{cd}	20.58 ± 1.75^{b}	23.86***
M. anisopliae	2.48 ± 0.23^{cd}	6.48 ± 0.61^{c}	15.02***
M+B	2.55 ± 0.25^{cd}	6.60 ± 0.66^{c}	14,05***
M+A	2.35 ± 0.18^{d}	5.08 ± 0.62^{c}	10,35***
A+B	3.75 ± 0.36^{cb}	16.38 ± 1.35^{b}	22,14***
M+A+B	2.35 ± 0.17^{d}	5.35 ± 0.72^{c}	9,93***
Decis	2.58 ± 0.33^{cd}	1.83 ± 0.23^{c}	4,56**
F	26.87***	30.66***	
Means (Df=387.67)	3.49±0.13	12.06±0.69	12.07***

C: Negative control; A: *A. indica*; B: *B. dalzielii*; M: *M. anisopliae*; M+B: *M. anisopliae+B. dalzielii*; M+A: *M. anisopliae+A. indica*; A+B: *A. indica+B. dalzielii*; M+A+B: *M. anisopliae+A. indica+B. dalzielii* ; D: Decis; ns: p>0.05; *: p<0.05; **: p<0.001; ***: p<0.0001. For each cowpea variety values of the same column denoted by the same upper-case letter are not significantly different between treatments at the indicated level of probability (Student–Newman–Keuls test).

4. DISCUSSION

The higher thrips population density was observed on the B125 cowpea variety probably due to its short growing cycle which has promoted early flowering. In the Sudano-Sahelian agro-ecological zone, the rainy season is short duration (up to 3 months). Under these conditions, the dry season came too early and did not allow the Bafia variety to produce enough flowers with its intermediate cycle longer than that of B125. It has been reported that the agroecological parameters can influence the blooming phase of cowpea varieties (Dugje *et al*. 2009). Regarding the action of natural insecticides, the reduced efficacy of *A. indica* extract is similar to the observations of Barry *et al*. 2017, confirming the argument that neem-based products could be more effective in storage than in the field (Bambara and Tiemtore 2008). The higher efficacy of *B. dalzielii* extract is due to the adhesive factors such as a gum found in *B. dalzielii,* which makes it viscous (Younoussa 2016), to improve its efficiency. According to Mfuti *et al*. (2021), adhesion is an important factor of the effectiveness of a treatment. The efficiency of *M. anisopliae* treatment may have been boosted by kerosene, which has insecticidal properties too (Djouaka *et al*. 2007). Kerosene and cotton seed oil have shown good adhesion to promote adequate application (Mfuti *et al*. (2021) for direct contact and efficacy on thrips as pointed out by Seye *et al*. (2012), who indicated that *M. anisopliae* was more effective than neem. The fact that combination of *A. indica* and *B. dalzielii* treatments were more effective in the thrips population density reduction than single treatments could be explained by the increased synergetic effects of both treatments. For other combinations with *M. anisopliae*, the bioactivity was attributed to the presence of adhesive factors which is in agreement with other studies (Barry *et al*. 2019; Seye *et al*. 2012), which showed a better efficacy of *M. anisopliae* combined with neem rather than individual applications. *Metarhizium anisopliae* and other plant extracts used in this work have potential to act synergistically in reducing the thrips population. Decis, with its large spectrum and systemic action, was more effective than all natural insecticides. Several authors (Barry *et al*. 2019; Bambara and Tiemtore 2008; Ngakou 2007) have demonstrated the greater effectiveness of Decis than other treatments. The efficacy of different insecticidal treatments was more pronounced on larvae than on adult thrips; this could be explained by the fact that cuticle of larvae are still very weak, not hard to protect larvae as that of adult since being the primary protective barrier for insects (Benserradj 2014).

Concerning the reduction of damage on the cowpea leaves, the different treatments had a similar effect on B125 and Bafia cowpea varieties in both years of experiment. Kerosene and cottonseed oil promoted the dissolution of *M. anisopliae* spores (Djouaka *et al*. 2007; Mfuti *et al*. 2021) and render the leaves stiff enough. This makes leaves less prone to be perforated by thrips. In addition to the leaf stiffness, the viscosity of *B. dalzielii* previously mentioned reinforces the protection of cowpea crop against thrips infestation. This is in turn considerably suppressed the leaf damage when using the combination of *M. anisopliae*+*B. dalzielii*. All this would therefore enable this treatment to protect cowpea leaves as much as the Decis on B125 and Bafia varieties in 2014 and 2015. Combination treatments, through the synergy of their different constituents (Barry *et al*. 2017; Seye *et al*. 2012; Ngakou 2007) equally protected cowpea leaves as the synthetic insecticide Decis, and have been more effective than that of synthetic insecticide

5. CONCLUSION

The aqueous extracts of *A. indica* and *B. dalzielii* and the mycoinsecticide *M. anisopliae* alone or in combination were found effective to reduce pest population and crop damage. The aqueous extracts of *A. indica* and *B. dalzielii*, the mycoinsecticide *M. anisopliae* and their combination, could be recommended as component of integrated management of the cowpea flower thrips. These natural insecticides could cope well when applied on the early mature B125 cowpea variety than intermediary Bafia variety. Considering the conservation of environment, the insecticidal formulations tested in this study could supersede the commercial pesticides used in crop protection. However, further studies on these natural products concerning their effect on beneficial insects and their persistence need to be carried out in order to optimize their use and potentialize their protective effect.

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