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The incidence of aqueous neem leaves (*Azadirachta indica* A. Juss) extract and *Metarhizium anisopliae* Metch. on cowpea thrips (*Megolurothips sjostedti* Trybom) and yield in Ngaoundéré (Adamaoua-Cameroun)

Barry Borkeum Raoul, Ngakou Albert, Tamò Manuele and Nukenine Elias Nchiwan

Abstract
The ability of *Azadirachta indica* aqueous extract and the mycoinsecticide *Metarhizium anisopliae* interactions in controlling the cowpea thrips (*Megolurothips sjostedti)* was compared in the field grown *Vigna unguiculata* at Dang, Ngaoundere-Cameroon. The field trial was arranged in a completely randomized block design with five treatments, each of which was replicated four times. The five treatments included the control and the four tested insecticide products. *V. unguiculata* plants were sprayed three times at flowering stage with the insecticide products at five days interval. The parameters taken into account were adult thrips counts after three sprays, the number of cowpea plant ramifications, the number of dry pods, and the weight of dry grains at harvest. All the tested insecticides significantly reduced the *M. sjostedti* population, with the efficiency grade *A. indica* < *M. anisopliae* < *A. indica* + *M. anisopliae* < Decis®. Insecticides applications also increased *V. unguiculata* grain yields, with effectiveness similar to that of thrips populations, although Decis® and the combination *A. indica* + *M. anisopliae* had the same effect. There was a strong inverse linear correlation between grain yield and thrips population size (R^2 = 0.96). *M. anisopliae* and *A. indica* induced more ramifications in *V. unguiculata* than Décis® and the combination *A. indica* + *M. anisopliae*. Our results suggest that the combination *A. indica* + *M. anisopliae* could be considered as a potential insecticide in the management of thrips in *V. unguiculata* fields. This would increase *V. unguiculata* grain yields, alleviate hunger and malnutrition as well, and reduce environmental impact of residual synthetic chemical insecticides such as Decis®.

Keywords: *Azadirachta indica*, *Metarhizium anisopliae*, *Megalurothrips sjostedti*, *Vigna unguiculata*, grain yield

Introduction
More than half the African population including Cameroon is leaving from agriculture which is an important element to be taken into consideration in the development, as far as food production is concerned (Adeoti *et al*., 2002) [1]. View in this way, agriculture needs yield improvement, not only in quantity, but also in quality, while preserving the environment. Hence, valorisation of multipurpose crops with diversified incomes such as cowpea is to be promoted (Anku-Tsede, 2000) [5].

Vigna unguiculata L. (Walp.) occupies an important role in the diet of the guinea-savannah and sudano-sahelian population (Isubikalu *et al*., 2000) [15]. This legume is rich in nutritious protein (20-25%) (Bressani, 1985; Rivas-Vega *et al*., 2006) [8, 32] and contain calories and protein more than millet or sorghum (Ndiaye, 1996) [25]. The leaves are used in various dishes (Nielsen *et al*., 1997) [28], while the whole plant serves as feedstock for rearing animals, or as green manure in agriculture (Ta'ama, 1986; Jayathilake *et al*., 2018) [40, 17]. Cowpea also improves soil fertility through biological nitrogen fixation (Ngakou, 2007) [26]. With these attributes, cowpea cropping could then be a major tool not only for the equilibrium of one diet, but also for the economic development of a country (Adeoti *et al*., 2002) [1].

However, cultivation of cowpea is facing several problems such as fungal, bacterial and viral diseases (Singh *et al*., 1997) [37], in addition to insect pests that cause damages to crops and considerable yield loses (Tamò *et al*., 1993) [42].

In fact, cowpea is attacked at all its developmental stages and from the field to storage (Akingbohungbe, 1982) [3]. The potential damaging insect pests in the field are the flower thrips *Megalurothrips sjostedti* Trybom (Thysanoptera, Thripidae), the pod borer *Maruca vitrata* Fabricius (Lepidoptera, Pyralidae), and the brown aphids *Clavigralla tomentosicollis* Stal (Heteroptera, Coreidae) (Singh and Jackai, 1985) [39]. Among these pests, *M. sjostedti* is the first to invade the plant (Taylor, 1974). It causes necroses and total destruction of flowers or flower bugs, leading to the yield reduction from 20 to 70% (Singh and Allen, 1980; Rusoke and Rubaihayo, 1994; Edema and Adipala, 1996) [38, 33, 11]. It appears necessary to combat these insect pests, and particularly *M. sjostedti* in order to reduce the attributed yield losses.

Previous works have revealed cowpea yield increment as the response of synthetic insecticides uses (Alghali, 1992; Kyamanywa, 1996; Parh, 1999; Karungi *et al.*, 2000) [4, 20, 31, 18]. Without protection, thrips will considerably reduce the yield of this crop (Ezueh, 1981; Jackai and Daoust, 1986; Sabati *et al.*, 1994) [13, 16, 34]. However, the use of synthetic insecticides has often caused problems than it has solved (Bambara and Tiemtore, 2008) [6]. They are not only costly, but also increase the development of resistance to target insect pests (Immaraju *et al.*, 1992; Margni *et al.*, 2002) [14, 21] and they destroy the environment (Ouedraogo, 2004) [30]. To overcome these negative impacts, the development of friendly, safe and ecological alternative strategies could be important to enhance the pest control, and thus, to improve the crop yields.

Some of these strategies have included several plant extracts used as natural plant insecticides, or chemical insecticide substitutes. Under these perspectives, the efficacy of neem has been demonstrated (Belanger and Thadee, 2005) [7], and on the other hand, the enthomophagous fungi *Metarhizium anisopliae* has been shown its insecticidal potential (Tamò *et al.,* 2003; Ngakou *et al.,* 2008) [41, 27].

The main objective of this research was to find out if the interaction neem leaves extract and the enthomophagous fungi *M. anisopliae* could cope with the reduction of *M. sjostedti* population, thereby improving cowpea yield in the field. The outcomes from this study could boost our knowledge on the control measure to be taken against the cowpea flower thrip *M. sjostedti*.

Material and Methods

Experimental design and treatments

Experiments were carried out in the guinea-savannah agro-ecological zone during the cropping season extending from August to December 2012. The experimental field was prepared on a (35×14) m^2 flat surface area, and was organized in a Completely Randomised Block Design (CRBD), in which each of the four blocks was separated 1m apart, and was made up of 5 repeated treatments with (2.25×4.5) m^2 as experimental unit. The five treatments were: T1 for negative control with zero application, T2 for neem extract or plant insecticide, T3 for *M. anisopliae* or mycoinsecticide, T4 for neem extract and mycoinsectide, and T5 for the synthetic insecticide Décis® or positive control. The plant based insecticide and the mycoinsecticide were applied by spraying with three different hand-held sprayers (APPROX) corresponding to T2, T3 and T4, in order to avoid contamination. Each treatment was applied thrice with 75 ml solution at 5 days interval, 60, 65, and 70 days after planting (DAP).

Biological material and formulations

Cowpea seeds used were those of the local Bafia variety with a long flowering period that allows better flower collection, as previously described by Ngakou (2007) [26]. Seeds were sown at 75 cm between and 50 cm within the lines.

Neem extract was obtained following the extraction method described by SPS (Technical file 2), according to which 5 L neem solution could be prepared from 1 kg leaves, thus 80 kg of neem leaves to apply approximatly on 1 ha field. In the laboratory, 500 g neem leaves harvested from neem trees in Ngaoundere was pounded in a mortar. The pounded leaves were mixed with 2.5 L tape water in a bucket, and sat for 12 h for maceration, after which neem leaves were removed from the mixture and the extract filtered through a 0.4 mm tissue mesh. The final solution used in the field was diluted at 10% (v/v) with tape water, since treatment started before thrips attack's. *M. anisopliae* strain ICPE 69 was obtained from the Department of Plant Health Management of the International Institute of Tropical Agriculture (IITA), Cotonou Benin. *M. anisopliae* solution was formulated as previously described (Ngakou *et al.,* 2008) [27], by disolving 25 g of *M. anisopliae* in mixture of 350 ml kerosene and 150 ml groundnut oil. The synthetic insecticide used as positive control was the large spectrum insecticide Decis®, pursued from a phytosanitary store in Yaounde-Cameroon, and prepared by diluting 3 ml of Décis® in 15 L tape water as indicated by the Manufacturer. Each of the insecticide receipt was sprayed at a rate of 75ml/experimental unit, 3 times in 5 days apart.

Assessment of plant and thrips parameters

The number of thrips per cowpea flower was evaluated at flowering stage on 5 days after insecticide spray by dissecting flowers and counting adult insects under a stereomicroscope (Academy Glass Magnifier, Ø:100 mm), on 20 randomly selected flowers sampled per treatment. The number of ramifications, as well as the number of pods was assessed each on 20 randomly selected plants per treatment. At harvest, the seed yield was evaluated and expressed in mg/treatment (Ngakou *et al.*, 2008) [27]. Number of ramification were assesed by counting boughs on plant stem (N'gbesso *et al*., 2013) [23].

Statistical analysis

Data on thrips population were first transformed into square values to reduce errors on variance. Then all data were submitted to Analysis of Variance (ANOVA), while differences between treatments for a particular parameter were discriminated using the Least Significant Difference (LSD) test of Fisher at 5% level. Correlations between parameters were brought out and data expressed in graphs, were plotted using Microsoft Office Exel 2007.

Results

When different types of insecticides were applied, the plant based insecticide neem induced a little reduction of the population of thrips in flowers compared to *M. anisopliae* alone and the association neem + *M. anisopliae* (Figure 1). The synthetic insecticide was very effective in decreasing the population of thrips with only 3 individual per flower.

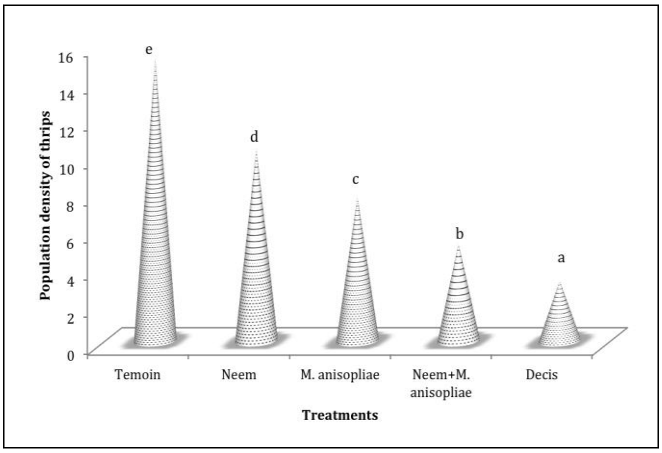

Fig 1: Population density of thrips as influenced by different insecticide receipts.

All the insecticides induced increased number of cowpea ramifications compared to the negative control (Figure 2), with treatment *M. anisopliae* and neem + *M. anisopliae* having more effect over the other treatments. Treatment Decis® as positive control did not have an effect on the development of ramifications in cowpea.

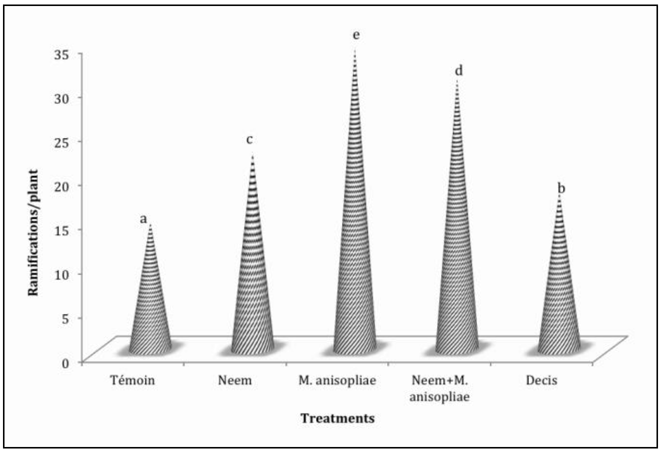

Fig 2: Changes in cowpea ramifications per plant as affected by insecticide treatments

At harvest, the number of cowpea pods produced from *M. anisopliae* sprayed plants was the most elevated per plant (8) compared to the synthetic insecticide Decis® or neem (3) alone, and the association neem + *M. anisopliae* (Figure 3).

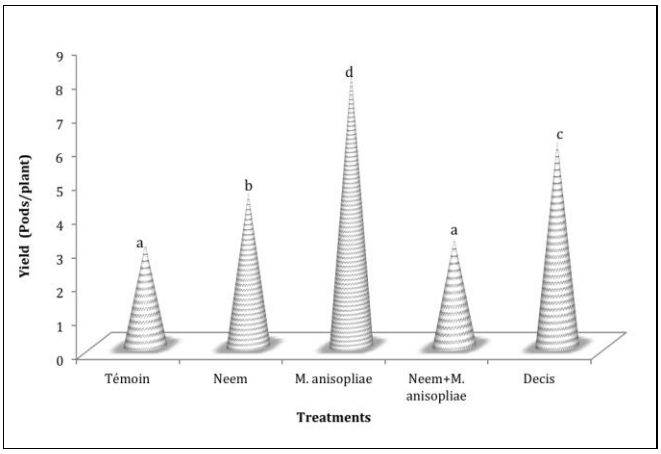

Fig 3: Variation of cowpea pods at harvest as influenced by insecticide treatments

Cowpea yield at harvest expressed as plant seed dry weight per treatment (mg) was very much affected by the type of insecticide sprayed (Figure 4). Treatment neem was revealed as the treatment producing the lowest seeds' weight (81.49 mg), although it increased the seed biomass by 1.81% compared to the negative unsprayed control. *M. anisopliae* treatment was more effective than neem treatment, but lesser than neem + *M. anisopliae* sprayed plants wich produced similar seed weight on average than that of the positive control Decis® (199.60 mg).

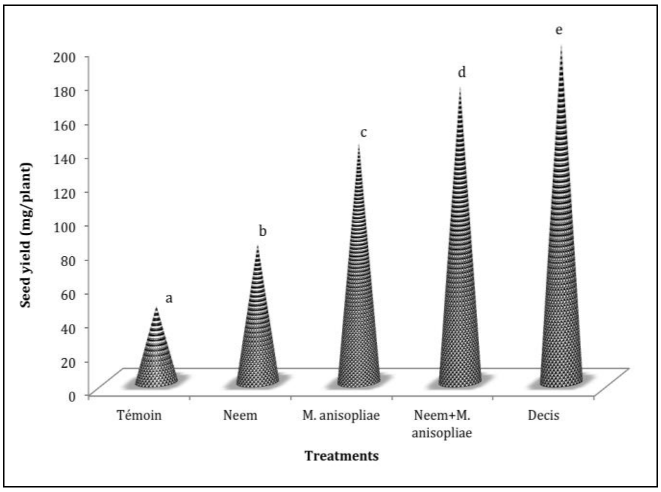

Fig 4: Changes in cowpea seed yield at harvest in different insecticide treatments.

When the population dynamic of thrips surveyed on 5 days was assessed (Figure 5), the number of adult thrips of the negative control plot was always above that of the others treatments. By ranking the synthetic insecticide, Decis® was the most effective in maintaining the thrips' population low, followed by treatments neem + *M. anisopliae*, and neem alone. All treatments had the same curve shape, with the exception that the pick of control treatment occurred one day before that of the other treatments.

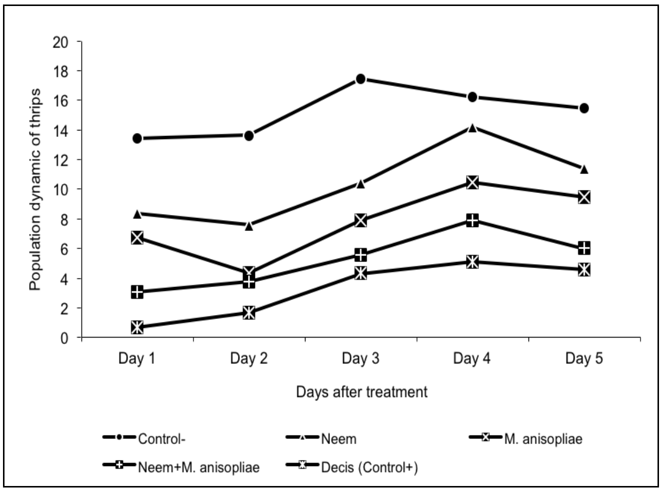

Fig 5: Population dynamic of cowpea flower thrips as influenced by different insecticide receipts.

Table 1 indicate that only the parameter population density of thrips and cowpea yield were correlated, but negatively. Treatments that had few insects were revealed to produce more seeds yield at harvest. In other words, the less the population of thrips, the more elevated of yield of a treatment.

These two parameters were negatively linked, but significant correlated ($p<0.01$; r = 96.32). The regression plot between cowpea yield and the population density of thrips is illustrated on Figure 6.

Table 1: Correlation between cowpea plant and the field parameters

	Thrips	Yield	Ramifications	Pods
Thrips	1			
Yield	-0.98**	1		
Ramifications	-0.42	0.41	1	
Pods	-0.41	0.40	0.45	1

**$p<0.01$; dl=3

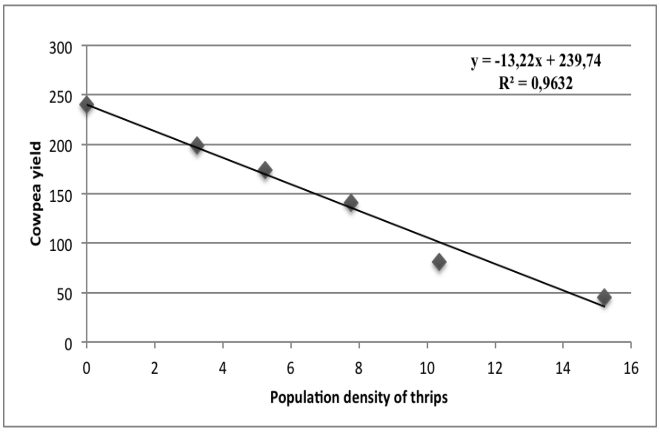

Fig 6: Regression plot between cowpea yield and the population density of thrips.

4. Discussion

All insecticides used in this research had an impact on thrips population density. *M. anisopliae* ICIPE69 wich better acted on thrips population than neem, contributing to reducing the thrips by 50%. In a similar experiment conducted in the field in western Kenya, *M. anisopliae* resulted in a reduction of thrips population by 33-49% (Ekesi *et al.,* 1998) [12], which lines with findings of this research. In fact, the effect of *M. anisopliae* as treatment might have been boosted by carrier components such as kerosene and groundnut oil which insecticidal properties have recently been reported (Djouaka *et al.,* 2007) [10]. Despite these attributes, the mycoinsecticide *M. anisopliae* acted less than the chemical insecticide Decis®, which has been proven to extend its activity not only on thrips, but also on numerous other devastating insects (Mouffok *et al.,* 2008, Ngakou *et al.,* 2008) [22, 27]. Moreover, the relatively low effectiveness of *M. anisopliae* compared to Decis® has rather been attributed to its slow action (Kassimatis, 2000) [19]. The combination neem + *M. anisopliae* was better than each of *M. anisopliae* or neem alone on thrips, with an efficiency closer to that of Decis®. This combined treatment might hold its main efficiency from the synergistic effect of both the neem and *M. anisopliae* in reducing the thrip density. These results are in agreement with those obtained by Sharififard *et al.,* (2011) [36], who revealed that the combination of *M. anisopliae* and Spinosad was more efficient in controlling *Musca domestica* than each treatment considered alone.

As far as the number of ramifications is concerned, the synthetic insecticide Decis® had the lowest positive effect on the quantity of ramifications compared to other insecticides. Its acting time was shorter than that of other treatments. Neem induced production of more ramifications than the insecticide Decis®. Neem has been reported not only to fight against devastating insects through its repellent effect, but also against plant diseases (Belanger and Thadee, 2005; Bambara and Tiemtore 2008) [7, 6] by increasing the development of ramifications, thus plant growth. *M. anisopliae* treated plants also produced more ramifications than others due to its long lasting effect on plants in the field. It has been reported to infect more than 100 insect species, including fruit flies, root soil insects (Bruck, 2005; Thamarai *et al.,* 2011) [9, 44]. The association neem + *M. anisopliae* was more efficient in producing ramifications than *M. anisopliae* alone. This mixture benefits from both neem and *M. anisopliae* properties enough to booster plant growth through increased ramifications.

The variation of ramifications with insecticides type obviously induced that of pods at harvest. Despite its efficacy on thrips, the combination neem + *M. anisopliae* was less effective on pods production, which might be related to reduce the quantity of neem used. Having a positive effect on plant health (Belanger and Thadee, 2005) [7], the reduction of neem quantity in the mixture may have affected its efficacy, since neem alone as treatment contributed to enhanced pod production than the combination of neem + *M. anisopliae*. *M. anisopliae* induced not only more ramifications production, but also, more pods production, relative to its wide spectrum of action and its long lasting effect in the field (Bruck, 2005; Thamarai *et al.,* 2011) [9, 44], which allows improved plant growth. It is well recognized that the more ramifications a plant will have, the more chances it will produce flowers and pods. However, producing a lot of pods do not necessary mean high seed yield. Among the insecticide receipts, treatment neem produced the lowest seed yield as the results of numerous damages inflicted to the host plant. Such findings were revealed by Bambara and Tiemtore (2008) [6], who investigated and compared the impact of neem, *Euphorbia balsamifera, Hyptis spicigera* and Decis® on cowpea insects in Burkina Faso.

Although *M. anisopliae* was better than neem as far as seed yield is concerned, its efficacy was below that of synthetic insecticide Decis®, being the insecticide with a large spectrum on diverse insects (Nampala *et al.,* 1999; Adipala *et al.,* 2000; Isubikalu *et al.,* 2000; Oparaeke *et al.,* 2005) [24, 2, 15, 29].

Decis® was the most effective insecticide despite the decrease of its efficacy with time, compared to *M. anisopliae* that acts slowly (Kassimatis, 2000) [19], but persisted in the field, whereas the most convenient treatment was the combination of neem + *M. anisopliae.*

The highest correlation between thrips population density and yield could be attributed to the fact that thrips destroy cowpea flowers, which are the reproductive organ of the plant. In fact, 96.32% of cowpea yield was related to thrips density, and according to the regression slop, yield would decrease by 13 g, when the thrips population density is increased by one individual. The damages inflicted to the host plant during its feeding period would lead to necrosis and/or premature abortion of flower bugs and flowers, with the yield losses ranging between 20 and 70% (Singh and Allen, 1980; Rusoke and Rubaihayo, 1994; Edema and Adipala, 1996) [38, 33, 11].

5. Conclusion

This research has highlighted that the combination of neem + *M. anisopliae* is the treatment that can substitute the synthetic insecticide Decis® in improving cowpea production through increased plant growth and yield. This treatment has a long lasting effect in the field and its effectiveness is almost equal to that of Decis®. Therefore, a sustainable management of cowpea production should consider the aforementioned combined treatment that could be recommended to biologically control cowpea insects, and particularly the thrips *Megalurothrips sjostedti* in the field.

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