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Table of Contents: Volume 6 Number 11, November, 2014

## ARTICLES

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*Full Length Research Paper*

# Evaluation of the efficiency of *Gaeolaelaps aculeifer* in control of plant parasitic nematode *Tylenchulus semipenetrans* under greenhouse conditions

**Aazam Salehi[1]*, Hadi Ostovan[1] and Mohamad Modarresi[2]**

[1]Department of Entomology, College of Agricultural Sciences, Shiraz Branch, Islamic Azad University, Shiraz, Iran.
[2]Department of Plant Breeding, College of Agriculture and Natural Resources, Persian Gulf University, Bushehr, Iran.

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**Biological control of plant parasitic nematode *Tylenchulus semipenetrans* was studied under greenhouse conditions. In the present study, the effect of the soil-dwelling predatory mite, *Gaeolaelaps aculeifer* (Acari: Laelapidae), on the population development of citrus nematode was examined. Compared to the nematode-alone, all mite treatments significantly restricted reproduction of citrus nematode. Nematode population ranged from 126 to 161 J2/100 cm$^3$ soil for the mite-treated plants compared to 398.25 J2/100 cm$^3$ soil for the nematode untreated plant. As a result, *G. aculeifer* significantly reduced citrus nematode *T. semipenetrans* populations under greenhouse conditions.**

**Key words:** Acari, Biological control, Laelapidae, Predatory mite.

## INTRODUCTION

Plant parasitic nematodes are widespread and cause serious losses to most agricultural crops (Al-Rehiayani and Fouly, 2005). Nematodes are often managed with chemical nematicides which can contaminate agro-ecosystems. Natural antagonistic of nematodes and biocontrol agents may provide an alternative to the use of pesticides for nematode management. Numerous nematode species are associated with the citrus rhizosphere; however few species are known to be of economic importance (El-Banhawy et al., 1997). Many nematode species have been reported to be parasiting the citrus but *Tylenchulus semipenetrans* (Cobb, 1913) was the most important on worldwide basis (Safdar et al., 2010).Citrus nematode is one of the most important root nematodes of plant trees that have worldwide distribution and cause reduction of crop production and vegetative growth. In addition, this nematode creates slow decline of citrus trees (Ayazpour et al., 2010). Yield reduction by citrus nematode depending of the infection rate, but on average is 10 to 30% (Verdego-Lucas and McKenry, 2004).

Methods commonly employed to control *T. semipenetrans* depend on local conditions and focus on excluding the pest, minimizing losses through crop management and reducing population of the parasite using nematicides or resistant root stock (El-Banhawy et al., 1997). Considerable information available in the literature has documented the effectiveness of several

*Corresponding author. E-mail: aazam.salehi@yahoo.com.

biological agents to manage plant parasitic nematodes (Al-Rehiayani and Fouly, 2005). The majority of mesostigmatid mites in soils are general predators which prey on a range of invertebrates including nematodes (Walter and Lindquist, 1989), although, the minority, are specialized predators feeding on nematodes (Sharma 1971; Habersaat, 1989). Previously, it was found that nymphs and adults of *Lasioseiuss scapulatus* (Kennet) had the ability to capture, consume and complete its entire life cycle on the root knot nematode *Meloidogyne incognita* (Kofoid and White Chitwood) (Imbriani and Mankau, 1983).

Also, the suitability of egg masses of *Meloidogyne* spp. As food source of the ascid mite species *Lasioseius dentatus* (Fox) was studied where it was found that the predatory mite successfully completed its whole life span on egg masses (Fouly, 1997). Al-Rehiayni and Fouly (2005) was studied effect of adding *Cosmolaelaps simplex* (Fox) or aldicarb for control of *T. semipenetrans* on citrus seedlings in greenhouse.

On the other hand, the mesostigmatid mites family Laelapidae Berlese is considered one of the most important groups of soil predators, where it usually feeds on nematodes (Muma, 1975). Predatory laelapids tend to be voracious, polyphagous predators that reproduce quickly and can be reared easily (Beaulieu, 2009). This makes them good candidates for biological control of pests that spend time in the soil or in other plant growing media. The genus *Gaeolaelaps* Evans and Till is currently one of the largest genera of the family Laelapidae. Some species of this genus, such as *Gaeolaelaps aculeifer* (Canestrini), *G. oreithyiae* (Walter and Oliver (1989), and *G. gillespiei* Beaulieu, are aggressive predators of nematodes and immature arthropods (Kavianpour et al., 2013).

*Gaeolaelaps* was considered at different taxonomic levels by authors: as a species group (Van Aswegen and Loots, 1970); or as a subgenus of *Hypoaspis*sens. lat. (Karg 1989; Faraji et al., 2008), and as a distinct genus (Lapina, 1976; Hyatt, 1964).

In the present study, we investigated the efficiency of soil-dwelling predatory mite *G. aculeifer* in biological control of citrus nematode in greenhouse and the effect of adding mite individuals for reduce *T. semipenetrans*on citrus seedlings.

## MATERIALS AND METHODS

### Rearing of predatory mite in laboratory

The predatory laelapid mite *G. aculeifer* was isolated from soil samples under shrubs of hopbush, *Dodonaea viscose (L.) Jgacq.* in Fars Science and Research University. After identification, isolated mites were reared in glass jars filled with damp sawdust. Mite samples have been maintained on the acarid mite species *Rhizoglyphus robini* Claparede, as a food source in rearing units. The acarid mite was maintained in laboratory on onion. All units were kept under normal room temperature at 25 ± 1°C and 65 ± 5% relative humidity.

### Extracting nematode *T. semipenetrans* in laboratory

Nematodes were extracted from soil samples taken with a hand trowel at 15-30 cm beneath the tree canopy in citrus orchard. In this study, Baermann's technique was used to separating nematode of soil suspention (Goody, 1957).

### Greenhouse experiments

A group of 20 plastic pots (25 cm in diameter) containing sand loam sterilized soil and previously transplanted with single 3-month seedlings of key lime (*Citrus aurantifolia).* The transplanted pots were divided into five groups, four pots each, where the first group was infected with approximately 100 juvenile stages of *T. semipenetrans*/pot. The second group received the mites at the same time of nematode inoculation, while the third one received 20 individuals of *G. aculeifer*/pot 15 days after nematode inoculation. The fourth group was infected with nematodes 15 days after adding 20 individuals of *G. aculeifer*/pot and the fifth one was left without any nematode inoculation and mites. All pots were kept under greenhouse conditions at 27 ± 1°C and 65 ± 5 % relative humidity.

Ninety days after nematode inoculation, citrus seedling that were carefully under consideration where data dealing with shoot length and fresh weight for root system were recorded. Moreover, the second juvenile stage (J2) found in 100 mL of soil samples was estimated for each nematode treatment after extraction by sieving using modified Baermann-funnel method (Goody, 1957). On the other hand, soil samples of about 250 g each were also subjected for mite extraction by the aid of the modified Berlese's (Tullgern's) funnels (Berlese, 1905). Where, the average number of mite/250 g of soil was calculated.

In all cases, data were subsequently analyzed by least significance difference (LSD), Duncan's multiple rang and analysis of variance (ANOVA) testes, where the reproduction index of predatory mite = final mite population (PF)/ initial mite population (PI).

## RESULTS AND DISCUSSION

In the study of plant growth, the mite-treated citrus plants showed the better growth as compared to the untreated plants. Significantly enhanced shoot growth was observed in all the mite-treated citrus plants as compared to the *T. semipenetrans* untreated control (35.70 cm) (Table 1).

Root weight was slightly enhanced in all mite-treated plants but this response was not significant compared to the plants with nematodes only. The citrus plants without nematodes had the greatest shoot length (58.1 cm). Seedlings with nematode alone as compared to the citrus plants without nematodes showed symptoms such as small and yellowing leaves, low growth, and death of top of branches and defoliate.

Based on the fact that the *T. semipenetrans* is semi-parasitic nematode; therefore predatory mite feeds on egg masses and juvenile stages of *T. semipenetrans*. Similarly, it was found before that addition of the predatory mite species *L. dentatus* either at the same time or 40 days after root-knot nematode inoculation had significant improvement in shoot length, weight and root weight of tomatoes seedlings under greenhouse condition (Mostafa et al., 1997).

**Table 1.** Plant response after the infection of citrus nematode *Tylenchulus semipenetrans* in the presence of the predatory mite *Gaeolaelaps aculeifer* under greenhouse conditions.

| Treatment | | Shoot length (cm) | Root weight (g) |
|---|---|---|---|
| 1 | Nematode alone | 35.70$^{c}$ | 19.20$^{ab}$ |
| 2 | Nematode + mite at the same time | 42.70$^{b}$ | 20.21$^{a}$ |
| 3 | Mites 15 days after nematode inoculation | 37.45$^{b}$ | 18.70$^{ab}$ |
| 4 | Mites 15 days before nematode inoculation | 40.12$^{b}$ | 19.30$^{ab}$ |
| 5 | No mite and nematode inoculation | 58.10$^{a}$ | 21.04$^{a}$ |

*Means in a column followed by the same letter(s) are not significantly different (p=0.05).

**Table 2.** Citrus nematode *Tylenchulus semipenetrans* development in citrus seedlings in the presence of the predatory mite *Gaeolaelaps aculeifer* under greenhouse conditions.

| Treatment | | No. nematode/100 cm$^3$ soil (X) | No. mites/250 cm$^3$ soil (X) | Reproduction index of mites (PF/PI) |
|---|---|---|---|---|
| 1 | Nematode alone | 398.25$^{d}$ | - | - |
| 2 | Nematode + mite at the same time | 126$^{b}$ | 3.12$^{a}$ | 42.84$^{b}$ |
| 3 | Mites 15 days after nematode inoculation | 161$^{bc}$ | 2.44$^{a}$ | 31.51$^{ab}$ |
| 4 | Mites 15 days before nematode inoculation | 133.75$^{c}$ | 1.97$^{a}$ | 24.45$^{a}$ |
| 5 | No mite and nematode inoculation | - | - | - |

X= mean of 4 replicates, PF= extracted mite population, PI= initial mite population. *Means in a column followed by the same letter(s) are not significantly different (p=0.01) according to LSD test.

**Table 3.** Analysis of variance of treatments (ANOVA).

| S.O.V | df | Var. of no. nematode | df | Var. of no. mite | Var. of PF/PI |
|---|---|---|---|---|---|
| Treatments | 3 | 67465.17** | 2 | 1.3160** | 344.3933** |
| Std. error | 12 | 16.125 | 9 | 0.0676 | 16.5972 |
| cv | - | 1.96 | - | 10.35 | 12.37 |

**= The test has been significant at 1% level

The result shows the potential of the predatory mite *G. aculeifer* in repressing *T. semipenetrans* reproduction. Compared to the nematode alone, all mite treatments significantly restricted reproduction of *T. semipenetrans*. Nematode populations ranged from 126 to 161 J2s/100 cm$^3$ for the mite-treated plants compared to 398 - 25 J2s/100 cm$^3$ for the nematode untreated control (Table 2). Nematode population significantly was at lowest level when the predatory mites were added to the treatments at the same time of nematode inoculation.

In spite of a difference means of treatments 2 and 4 in LSD test did not have any significant differences, but tow treatments 1 and 3 showed significant difference (p=1%) according to LSD (Table 3).

Concerning the reproduction index of *G. aculeifer*, it was noticed that it was at its highest level when the predatory mites were added to the treatments at the same time of nematode inoculation (PF/PI=42.84) and followed by 31.51 and 24.45 for mites that were added 15 days after and before nematode inoculation, respectively (Table 2).

Also, there were no significant differences between the effect of application time of mites added to citrus seedlings 15 days after nematode inoculation and either mites added at the same time or mites added 15 days before inoculation n the reproduction index of *G. aculeifer*; whereas, there was significant difference between the effect of adding mites at the same time of inoculation and those that were added before inoculation on the reproduction index of *G. aculeifer*.

Similarly, it was previously found that the reproduction index of the ascid predatory mite *L. dentatus* was higher when mite individuals were added to tomato seedlings 40 days after root-knot nematode inoculation (Abou Setta et al., 1986) and similarly, these results significant match with findings (Al-Rehiayani and Fouly, 2005) about repro-

duction index of the predatory mite species *C. simplex* on *T. semipenetrans* under greenhouse condition. That may be due to mite species and its feeding behavior as well as to the biological aspects of nematodes. Therefore, it can be concluded that *G. aculeifer* had a better response which directly represented by its reproductive potentiality and capability to reducing citrus nematode populations when it was added at the same time of nematode inoculation. In other word, the predatory mite *G. aculeifer* had the chance to search the developmental individuals of citrus nematodes and feed on them before they can reach the root system and become more difficult for the predator. These results are in harmony with the previous findings where it was found that the developmental stages of root-knot nematodes were eaten by *L. scapulatus* under laboratory and greenhouse conditions (Imbriani and Mankau, 1983).

Finally, it can be concluded that the predator mite *G. aculeifer* could be considered as a biological control agent, which may limit populations of citrus nematode. Moreover, mite capability to feed, survive and reproduce on nematodes can be integrated with other control tactics and further field work in this area is highly warranted.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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*Full Length Research Paper*

# Effect of infection by *Metarhizium anisopliae* isolate ICIPE 51 on developmental stage, fecundity and intrinsic rate of increase of *Rhopalosiphum padi* and *Metopolophium dirhodum*

**Patrick Murerwa[1]*, Peter Futi Arama[2], Alice Wanjiku Kamau[1] and Nguya Kalemba Maniania[3]**

[1]Crops, Horticulture and Soils Department, Faculty of Agriculture, Egerton University, Nakuru, Kenya.
[2]Department of Crop Protection, Faculty of Agriculture, Rongo University College, Rongo, Kenya.
[3]Arthropod Pathology Unit, International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya.

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**This study assesses the pathogenicity of *Metarhizium anisopliae* isolate ICIPE 51 against different nymphal instars and adults of *Rhopalosiphum padi* and *Metopolophium dirhodum* and investigates effects of fungal infection on fecundity and intrinsic rate of aphid increase. To obtain different developmental stages, adult aphids were inoculated onto fresh leaf discs, reproducing parthenogenetically. Rearing was carried out to ensure different developmental stages were obtained at the same time so that treatments could be performed concomitantly. Concentrations of 1.0 x $10^6$, 3.0 x $10^6$ and 1.0 x $10^7$ conidia/ml were used for each developmental stage. Mortality was recorded daily for 10 days. For fecundity, treated aphids were transferred to a leaf in an assay cell, one aphid per cell and observed for 7 days. New born nymphs were removed after counting. Five to seven day adults were significantly more susceptible than nymphs of other developmental stages. No significant difference in susceptibility was observed within each stage in the first three days. Thereafter, susceptibility increased steadily to maximum levels of 71 and 57% for five to seven day old adults and 0 - 2 day old nymphs, respectively. *M. dirhodum* was significantly less fecund than *R. padi* at all concentrations. Fecundity and intrinsic rate of increase among both aphid species declined progressively over time. Thus, maximum fecundity of 3 and 3.5 nymphs/aphid among *M. dirhodum* and *R. padi* respectively was recorded during the first day as compared to less than 1 nymphs/aphid/day in each species from the sixth day. These results indicate that susceptibility of *R. padi* and *M. dirhodum* to entomopathogenic fungal control increases with aphid maturity and that both species are significantly more fecund in early adulthood, suggesting the stage as ideal for biopesticide management intervention.**

**Key words:** *Metarhizium anisopliae*, *Metopolophium dirhodum, Rhopalosiphum padi,* fecundity, intrinsic rate of increase.

## INTRODUCTION

Bird-cherry oat aphid, *Rhopalosiphum padi* (Linnaeus) and Rose Grain aphid, *Metopolophium dirhodum* (Walker) pose serious threat to bread wheat growers in Kenya. Both nymphs and adults suck plant sap and

cause serious damage right from the seedling to maturity stage. In addition, the most damage is caused by transmission of a number of viruses, especially *Barley yellow dwarf virus* (BYDV), for which the two species are the most important vectors (Riedell et al., 2003; A. Wangai, National Agricultural Laboratories, Kenya, personal communication).

A number of synthetic chemical insecticides have been used to reduce populations to below damage threshold level. However, large reproductive rates and wide range of host plants make aphids difficult to control (Borer et al., 2009). Moreover, concern about the hazardous effect of synthetic chemical insecticides on the environment and humans has prompted the search for more effective and safe control strategies (Sezen et al., 2004; Muratoglu et al., 2011). Entomopathogenic fungi (EPF) which have been reported to be pathogenic against a wide range of insect pest species including aphids (Purwar and Sachan, 2005) are among the strategies being considered. However, insect susceptibility to fungal infection is affected by a number of factors, such as the properties of the pathogen population, the host population as well as environmental conditions (Inglis et al., 2001). Among the host factors, host species, host age, the developmental stage and sex have been reported to affect host susceptibility to EPF.

Cereal-infesting aphids are multivoltine pests and individuals in all developmental stages are usually present on an infested wheat crop (Helmut and Richard, 2007). An understanding of the susceptibility of different developmental stages to fungal infection is important for the development of management tactics and will enable the optimization of the impact of biological control agents (Butt et al., 2001). A pathogen that is able to cause infection to more than one developmental stage of its host would be preferable to the one that is only pathogenic to specific stages, especially when the host insect has a high reproductive potential.

Entomopathogenic fungi have also been reported to affect fecundity and fertility in many arthropods, which may have implications for the population dynamics of the host (Quesada-Moraga et al., 2004). The possible reduction of reproductive potential of *M. dirhodum* and *R. padi* adults that are fungally challenged during oviposition may contribute to the overall efficacy of the treatment.

Results from previous screen house experiments identified *M. anisopliae* (Metschnikoff) Sorokin (Ascomycota: Hypocreales) isolate ICIPE51 as a potential candidate for management of *R. padi* and *M. dirhodum*. The present study therefore investigates the effects of infection by *M. anisopliae* isolate 51 on different developmental stages of *R. padi* and *M. dirhodum* as well as the effects of fungal infection on fecundity and intrinsic rate of natural increase of both aphids.

## MATERIALS AND METHODS

### Aphid rearing

*M. dirhodum and R. padi* were reared on wheat plants, *Triticum aestivum*, variety Mbuni in ventilated Plexiglas cages (60 x 35 x 70 cm) at temperatures between 24-28°C, 60-70% relative humidity (RH) and a photoperiod of 12:12 h (L:D) in a rearing room at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. The initial culture originated from aphids collected from Njoro town (0° 23'S and 35° 35'E), Kenya, in 2008. To obtain the different developmental stages for the experiments, adult aphids were collected from the aphid culture and put on fresh leaf discs placed on wet cotton wool in Petri dishes. The inoculated aphids reproduced parthenogenetically. Newly-emerged (one-day old) first-instar nymphs were transferred to new leaf discs and thereafter leaf discs were changed every four days. The rearing was carried out in such a way that different developmental stages were obtained at the same time so that treatments could be performed concomitantly.

### Fungal pathogen

*M. anisopliae* isolate ICIPE 51 was used in the study. It was sourced from the ICIPE's Arthropod Germplasm Centre and was selected because of its virulence against *M. dirhodum* and *R. padi*. The fungus was grown for 21 days on Sabouraud dextrose agar (SDA) plates at 26 ± 2°C. Conidia were harvested by scrapping the surface using a sterile rubber. Inocula were suspended in 10 ml sterile distilled water containing 0.05% Triton X-100 in universal bottles containing glass beads. Conidial suspensions were vortexed for 5 min to produce a homogenous suspension. Spore concentrations were determined using a haemocytometer. Viability of conidia was determined before each bioassay by spread-plating 0.1 mL of conidial suspension titrated at $3.0 \times 10^6$ conidia/mL on SDA plates. Sterile microscopic cover slip was placed on each plate and plates were incubated at 26 ± 2°C and examined after 15 h. Percentage germination was determined from 100-spore counts. Each plate was replicated four times. Over 94% of conidia germinated in all the tests.

### Inoculation of developmental stages

Nymphs aged 0-2 days, three to four days and adults (five to seven days old) were used in the bioassays. Both sides of fresh wheat leaves were sprayed with 10 mL of conidial suspension using Burgerjon's spray tower and allowed to dry for 20 min. Aphids were then transferred to the leaf discs in Petri dishes (90 mm diameter) using a camel hair brush. Concentrations of $1.0 \times 10^6$, $3.0 \times 10^6$ and $1.0 \times 10^7$ conidia/mL were used for each developmental stage. Control lots were treated with sterile distilled water containing 0.05% Triton X-100. Test-aphids were exposed to treated wheat leaf discs for 4 days, after which treated discs were removed and replaced with fresh and untreated leaf discs. Aphids were maintained in an incubator at 26 ± 2°C and 70-80% RH. Mortality was recorded daily for 10 days. Dead aphids were transferred to Petri dishes lined with moist filter paper to allow the growth of the fungus on the surface of the cadavers. Mycosis was confirmed by microscopic examination. Treatments consisted of 20 aphids each replicated five times and repeated twice.

*Corresponding author. E-mail: patrickmurerwa@gmail.com. Tel: +254-728-851685.

**Table 1.** Mean percent mortality of different nymphal instars and adults of *R. padi* and *M. dirhodum* treated with *M. anisopliae* isolate ICIPE 51.

| Stage | Mean mortality (%) | | | |
|---|---|---|---|---|
| | *M. dirhodum* | | *R. padi* | |
| | Treatment | Control | Treatment | Control |
| 0-2 day-old nymphs | 18.5[c] | 0.3[b] | 15.2[c] | 0.5[b] |
| 3 and 4 day-old nymphs | 21.8[b] | 0.8[b] | 20.0[b] | 0.6[b] |
| 5-7 days old adults | 31.1[a] | 2.4[a] | 25.6[a] | 2.0[a] |

Means within a column followed by the same letter are not significantly different at α = 0.05.

For fecundity bioassays, five replicates of three conidial concentrations (1.0 x $10^6$, 3.0 x $10^6$ and 1.0 x $10^7$ conidia/mL) were inoculated to groups each containing about 30 apterous adult aphids. This total included extra aphids to ensure that each treatment would have 20 live aphids after being treated with conidial suspension. Treated aphids were transferred to a leaf in an assay cell, one aphid per cell. Each assay cell consisted of a 60-mm transparent plastic Petri dish containing a 5-cm length of wheat leaf from a greenhouse-grown plant (2-3 week old) with the ends contacting bands of water-soaked, sterile cotton. The assay cells were maintained in the ventilated Plexiglas cages. The cotton wool in the Petri dishes was saturated daily with water and every three to five days aphids were transferred to new leaf disks. New born nymphs were removed after counting. The treated aphids were observed daily for seven days to record mortality and fecundity. The experiment was repeated twice.

#### Statistical analysis

Percentage mortality was normalized through angular transformation after correcting for natural mortality (Abbott, 1925). Mortality rates were separated across treatments using the ANOVA procedure of SAS (SAS Institute, 2003). Mean values were separated using LSD at 0.05 level.

Differences in fecundity and intrinsic rate of increase were tested by analysis of variance (Anova). The intrinsic rate of natural increase (rm) was calculated using the following formula as described by Wyatt and White (1977):

$$rm = \frac{0.74\ (\ln Md)}{d}$$

Where, Md is the number of nymphs produced over a period of time equal to that of the entire pre-reproductive period (d). This formula gives a good estimate of population growth rates in aphids (Dixon et al., 1993).

## RESULTS

### Susceptibility of different *M. dirhodum* and *R. padi* developmental stages to *M. anisopliae* isolate ICIPE 51

In the viability test, more than 94% of spores germinated. Control mortalities for 0-2 day-old nymphs, 3 and 4 day-old nymphs and 5-7 days old adults in both aphid species ranged between 0.3 - 0.5, 0.6 - 0.8 and 2.0 and 2.4%, respectively after 9 days post treatment. Table 1 shows the mortality caused by *M. anisopliae* isolate ICIPE 51 at different developmental stages among the two aphid species. There were significant differences among both aphid species observed in mortalities of all nymphal instars and adults ($P < 0.05$). Three and four day-old nymphs were significantly more susceptible than 0-2 day-old nymphs. The five to seven day old adults were the most susceptible stage with 31 and 25% mortality against *M. dirhodum* and *R. padi* respectively as compared to 18 and 15% for *M. dirhodum* and *R. padi* respectively registered among 0-2 day-old nymphs.

There were differences in aphid mortality among all stages with increasing concentration of *M. anisopliae* isolate ICIPE 51 and these differences were statistically significant (*P < 0.05*). The lowest mortalities for *M. dirhodum* and *R. padi* was 19 and 16%, respectively recorded at 1 x $10^6$ spores $mL^{-1}$ among the 0-2 day-old nymphs while the highest mortalities for *M. dirhodum* and *R. padi* was 51 and 44% respectively registered at 1 x $10^7$ spores $mL^{-1}$ among the 5-7 days old adults. Percent mortality of different nymphal instars of *M. dirhodum* and *R. padi* treated with different concentrations of *M. anisopliae* isolate ICIPE 51 is shown in Table 2.

*M. anisopliae* isolate ICIPE 51 was able to infect 3 and 4 day-old nymphs and 5-7 days old adults 48 h after treatment whereas 0-2 day-old nymphs recorded mortality after 72 h. 5-7 days old adults were the most susceptible taking between 6 - 7 days to register 50% mortality as compared to the 0-2 day-old nymphs which took the longest time of between 8 - 9 days. At the end of experiment, the lowest mortality of 57% and highest mortality of 71% were observed among the 0-2 day-old nymphs and 5-7 days old adults, respectively (Table 3)

### Dose and time effects of *M. anisopliae* isolate ICIPE 51 infection on the fecundity and intrinsic rate of increase of *R. padi* and *M. dirhodum*

#### *Dose effect*

Table 4 shows that the maximum fecundity in *M. dirhodum* and *R. padi* was 1.8 and 2.0 nymphs per aphid,

**Table 2.** Mean percent mortality of different nymphal instars and adults of aphids treated with different concentrations of *M. anisopliae* isolate ICIPE 51.

| Stage | Mean mortality (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | | Dose (Conidia/mL) | | | | | |
| | | | $1 \times 10^6$ | | $3 \times 10^6$ | | $1 \times 10^7$ | |
| | *M. dirhodum* | *R. padi* | *M. dirhodum* | *R. padi* | *M. dirhodum* | *R. padi* | *M. dirhodum* | *R. padi* |
| 0-2 day-old nymphs | $0.3^c$ | $0.5^b$ | $19.2^c$ | $16.2^c$ | $25.3^c$ | $20.4^c$ | $29.3^c$ | $23.6^c$ |
| 3 and 4 day-old nymphs | $0.8^b$ | $0.6^b$ | $20.7^b$ | $23.3^b$ | $28.7^b$ | $25.5^b$ | $36.9^b$ | $30.7^b$ |
| 5-7 days old adults | $2.4^a$ | $2.0^a$ | $31.4^a$ | $24.3^a$ | $39.2^a$ | $31.7^a$ | $51.4^a$ | $44.4^a$ |

Means within a column followed by the same letter are not significantly different at α = 0.05.

**Table 3.** Effect of time on mean percent mortality of different nymphal instars and adults of *R. padi* and *M. dirhodum* treated with *M. anisopliae* isolate ICIPE 51.

| Days after treatment | Mean mortality (%) | | | |
|---|---|---|---|---|
| | Control | 0-2 day-old nymphs | 3 and 4 day-old nymphs | 5-7 days old adults |
| 0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1 | 0.0 | 0.0 | 0.0 | 0.0 |
| 2 | 0.0 | 0.0 | 0.3 | 1.4 |
| 3 | 0.0 | 0.8 | 2.5 | 7.0 |
| 4 | 0.0 | 3.6 | 7.8 | 15.5 |
| 5 | 0.2 | 10.3 | 16.1 | 27.3 |
| 6 | 1.0 | 20.3 | 26.9 | 41.5 |
| 7 | 1.8 | 31.6 | 39.8 | 54.9 |
| 8 | 3.5 | 44.6 | 52.9 | 65.0 |
| 9 | 4.5 | 57.4 | 62.9 | 71.0 |
| LSD | | | 1.4 | |
| CV (%) | | | 24.3 | |

**Table 4.** Effect of different doses of *M. anisopliae* isolate ICIPE 51 on fecundity and intrinsic rate of increase of treated *M. dirhodum* and *R. padi*.

| Treatment | Fecundity | | Intrinsic rate of increase (rm), % | |
|---|---|---|---|---|
| | *M. dirhodum* | *R. padi* | *M. dirhodum* | *R. padi* |
| Control | $1.8^a$ | $2.0^a$ | $0.49^a$ | $0.54^a$ |
| $1 \times 10^6$ | $1.8^a$ | $2.0^a$ | $0.49^a$ | $0.55^a$ |
| $3 \times 10^6$ | $1.6^b$ | $1.7^b$ | $0.47^a$ | $0.48^b$ |
| $1 \times 10^7$ | $1.2^c$ | $1.4^c$ | $0.40^b$ | $0.47^b$ |

Means within a column followed by the same letter are not significantly different at α = 0.05.

respectively, as observed at the lowest concentration of $1 \times 10^6$ spores $mL^{-1}$. Both aphids species were significantly less fecund at $1 \times 10^7$ $mL^{-1}$, registering 1.2 and 1.4 nymphs per aphid for *M. dirhodum* and *R. padi*, respectively. There was no significant difference in fecundity among both aphid species between the control and $1 \times 10^6$ spores $mL^{-1}$ treatments. *M. dirhodum* was significantly less fecund than *R. padi* at all tested concentrations. The intrinsic rate of natural increase (rm) was different among the aphid species as well as among the treatments (*P < 0.05*).

The rm value was the highest at $1 \times 10^6$ spores $mL^{-1}$ (0.49 and 0.55 nymphs per aphid $day^{-1}$ for *M. dirhodum* and *R. padi* respectively) as compared to the lowest value of rm at $1 \times 10^7$ spores $mL^{-1}$ (0.40 and 0.47 nymphs per aphid $d^{-1}$ for *M. dirhodum* and *R. padi* respectively).

**Table 5.** Effect of time on fecundity and intrinsic rate of increase of *M. dirhodum* and *R. padi* infected with *M. anisopliae* isolate ICIPE 51.

| Days after treatment | Fecundity | | | | Intrinsic Rate of Increase (rm), % | | | |
|---|---|---|---|---|---|---|---|---|
| | *M. dirhodum* | | *R. padi* | | *M. dirhodum* | | *R. padi* | |
| | Treatment | Control | Treatment | Control | Treatment | Control | Treatment | Control |
| 1 | 3.0 | 3.3 | 3.5 | 3.7 | 0.82 | 0.87 | 0.91 | 0.96 |
| 2 | 3.1 | 3.5 | 3.2 | 3.7 | 0.67 | 0.71 | 0.69 | 0.74 |
| 3 | 2.6 | 2.7 | 2.8 | 3.0 | 0.53 | 0.56 | 0.55 | 0.58 |
| 4 | 1.5 | 1.9 | 1.8 | 2.1 | 0.43 | 0.45 | 0.45 | 0.46 |
| 5 | 0.7 | 0.9 | 1.0 | 1.2 | 0.30 | 0.33 | 0.36 | 0.40 |
| 6 | 0.3 | 0.5 | 0.3 | 0.5 | 0.23 | 0.28 | 0.30 | 0.33 |
| 7 | 0.1 | 0.1 | 0.1 | 0.2 | 0.20 | 0.25 | 0.26 | 0.29 |
| LSD | 0.1 | | | | 0.02 | | | |
| CV | 19.9 | | | | 10.8 | | | |

***Time effect***

There was a general progressive decline in fecundity over time in both aphid species (Table 5). Fecundity in the first 2 days among both species was more than 3 nymphs/aphid. Thereafter, fecundity at 4 and 7 days post treatment reduced significantly and respectively to 1.5 and 0.1 nymphs/aphid and 1.8 and 0.1 nymphs/aphid for *M. dirhodum* and *R. padi,* respectively.

The highest intrinsic rate of increase (rm) was recorded during the first day (0.82 and 0.91 nymphs/aphid/day for *M. dirhodum* and *R. padi* respectively) while the lowest (0.20 and 0.26 nymphs/aphid/day for *M. dirhodum* and *R. padi,* respectively) was recorded on the seventh day.

## DISCUSSION

Numerous studies indicate that aphids are susceptible to infection by diverse species of entomopathogenic fungi including *M. anisopliae* (Ibrahim et al., 2011; Shan and Feng, 2010). This study revealed that *M. anisopliae* isolate ICIPE 51 had pathogenic effects against *R. padi* and *M. dirhodum* although the latter was more susceptible with significant differences in mortality observed in all nymphal instars and adults. Susceptibility among both aphid species increased progressively with aphid age, 5-7 days old adults recording significantly higher mortalities than immature stages.

There are scant registers of the effects of *M. anisopliae* on developmental stages of either *R. padi* or *M. dirhodum*. However, it is possible to make comparisons with other insects. The higher susceptibility of adult aphids than immature 0-4 day old nymphs recorded in our study agrees with observation of Lopes and Alves (2011) that demonstrated adults of *Blattella germanica* (L.) (Blattodea: Blattellidae) were more susceptible to *M. anisopliae* infection than nymphs. Likewise, according to Romaña and Fargues (1992), the older larvae of *Melolontha melolontha* (L.) (Coleoptera: Scarabaeidae) were more susceptible to *Beauveria brongniartii* than the younger larval instars. Similar results have been reported by Ridsill-Smith and Annells (1997) who observed higher infection rate by *Neozygites floridana* in field-collected adults of *Tetranychus urticae* and *Halotydeus destructor* (Tucker) (Acarina, Penthaleidae) than in immature stages. In contrast, Haji et al. (2008) reported that fifth instar nymphs of Sunn pest were more susceptible to *B. bassiana* than adults. The foregoing reinforces an earlier observation by Ferron (1985) that relative susceptibility of different development stages of a host depends on the host species and on the fungal isolate. Ekesi and Maniania (2000) reported moulting to be an important factor in arthropod resistance to fungal infection, especially in arthropods with short ecdysis intervals. If the host is in an immature stage, molting could reduce the effectiveness of the fungal entomopathogen, in part owing to the shedding of conidia attached to the molted cuticle (Luz et al., 2003).

In our studies, germinated and ungerminated conidia were observed on the exuviae of *R. padi* and *M. dirhodum* following infection with *M. anisopliae*. It is probable the fungal inoculum was shed off with the exuvium following ecdysis leading to differential susceptibility observed in different nymphal stages and specifically the apparent decreased susceptibility of the immature aphid stages. The enhanced susceptibility of 5-7 days old aphids could as well be possibly attributed to the observed increased mobility of the mature adults across leaf surfaces as compared to the less active immature stages thereby increasing chances of contact of the relatively larger adult aphids with multiple fungal inocula.

Mortality in all life stages was dose-dependent, with the highest mortality occurring at $10^7$ conidia/mL. Comparable results were reported on *T. urticae* with *B. bassiana* (Saenz-de-Cabezirigaray et al., 2003). Similar dose-mortality responses on different developmental stages

have also been reported on many other arthropod pests (Feng et al., 1985; Ekesi and Maniania, 2000). According to our results, high doses and long periods (time) are required for *M. anisopliae* isolate ICIPE 51 to cause satisfactory levels of mortality.

This study showed that both *R. padi* and *M. dirhodum* infected by *M. anisopliae* sustained an increase in reproductive output in response to early stages of infection followed by a reduction 5 days post inoculation. In contrast, other studies have suggested that pea aphid, *Acyrthosiphon pisum* aphids infected by *P. neoaphidis* initially registered fast and sustained decline in fecundity (Baverstock et al., 2006). Studies assessing the alarm response of pea aphids infected with either *P. neoaphidis* or *B. bassiana* support the hypothesis that host-specific fungi like *M. anisopliae* modify the behavior of the host whereas more generalist fungi do not (Roy et al., 2005). Pathogen and host fitness are directly dependent on the number of viable offspring produced and it is predicted that both will be adopting strategies to maximize reproductive output. Many studies have demonstrated that a reduction in host fecundity can increase pathogen fitness as host resources such as energy are used by the pathogen for conidia production rather than by the host for reproductive output (Xu and Feng, 2002). In our study, the increase in aphid fecundity may thus have been a result of the host diverting resources to reproduction as a defense strategy to increase fitness and possibly ensure that part of their reproductive potential is realized. This may also benefit the pathogen through ensuring the continuation of a susceptible host population (Blanford and Thomas, 2001). The subsequent reduction in fecundity may be the outcome of an incidental process in which the indiscriminate invasion of host tissues and production of secondary metabolites interferes with nymph production. These hypotheses, however, require further exploration.

*M. anisopliae* isolate ICIPE 51 infection led to significant reduction of the host aphid's progeny in both species. Low levels of inocula ($10^6$ conidia/mL) of the entomopathogen appeared to have no significant effect on aphids' fecundity and intrinsic rate of increase. Baverstock et al. (2006) observed that infection of the pea aphid, *Acyrthosiphon pisum* by either *P. neoaphidis* or *B. bassiana* reduced the number of nymphs produced within 24 h of inoculation and over the entire infection period as compared to uninfected aphids. However, infection for 24 or 72 h did not alter the intrinsic rate of increase of the host aphid. Similar results to our study were observed in the reproductive output of *Tutta absoluta* (Pires et al., 2008) and *Diuraphis noxia* (Wang and Knudsen, 1993) using *M. anisopliae* and *B. bassiana*, respectively. Other studies that have shown comparable results on this topic include that on *Cylas puncticollis* (Ondiaka et al., 2008), *Anoplophora glabripennis* (Hajek et al, 2008) and *Megalurothrips sjostedti* (Ekesi and Maniania, 2000).

## Conclusions and recommendations

*M. anisopliae* isolate ICIPE 51 demonstrated pathogenicity against *R. padi* and *M. dirhodum* under controlled laboratory conditions. Virulence for all stages was dose-dependent and mortality increased with time. Low doses of the isolate appeared not to affect pre-lethal reproductive effects, such as fecundity and intrinsic rate of increase. Both aphid species were significantly more fecund in their early adulthood, suggesting the stage as ideal for biopesticide management intervention. These results showed that *M. anisopliae* isolate ICIPE 51 could be a viable alternative for control of *R. padi* and *M. dirhodum* in bread wheat.

On the other hand, it should be considered that the laboratory and greenhouse bioassays were conducted under optimal conditions for fungal growth (e.g., high humidity and constant temperatures and photoperiods), which are obviously very different from environmental conditions that would be encountered in the field (Butt and Goettel, 2000). Hence, additional research at field conditions to further evaluate and consolidate findings regarding biopesticide potential *M. anisopliae* isolate ICIPE 51 would be necessary.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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# Evaluation of cultivars and insecticides on insect pests and grain loss of rainfed cowpea (*Vigna unguiculata* (L.) Walp.) at Baga, Lake Chad shore area of Nigeria

**U. M. Maina[1*], B. M. Sastawa[2] and B. M. Biu[3]**

[1]Ramat Polytechnic Maiduguri, P.M.B. 1070, Maiduguri, Nigeria.
[2]Department of Crop Protection, Faculty of Agriculture, University of Maiduguri, P.M.B.1069, Maiduguri, Nigeria.
[3]Federal College of Education (Technical), Potiskum, Yobe State, Nigeria.

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**Field trials were conducted to determine effects of cultivar and insecticide application on grain yield and yield loss of cowpea to insect pest during the 2008 and 2009 cropping seasons at Baga (13° 29" N and 13° 32" E), Lake Chad shore area of Nigeria. Three cowpea varieties (Kanannado, Borno brown and IT98k-1312), two insecticides [cypermethrin (30 g) + dimethoate (250 g) and neem seed aqueous extract] and three spray regimes (one each at budding, flowering and podding) were evaluated for the control of pest on cowpea. The treatments were laid in a strip-split plot design and replicated three times each. The results reveal that flower thrips (*Megalurothrips sjostedti)*, Legume pod borer (*Maruca vitrata),* Blister beetle (*Mylabris* spp.) and Pod Sucking Bug (*Anoplocnemi scurvipes*) were the major insect pests of rainfed cowpea in the area. The variety Borno brown failed to produce flower in both seasons. IT98k-131-2 was more tolerant to damage by insect pests of budding, flowering and podding stages. Higher percentage increase in grain yield was achieved by three sprays of either Cymbush super EC (87.68 and 61.09%) or NSAE (81.85 and 53.69%) over control in 2008 and 2009, respectively. Pod damage of 22.3-26.3% was recorded in untreated control while in cowpea treated with Cymbush super EC and NSAE, pod damage was 7.0-7.4 and 8.8-10.6%, respectively. Grain yield loss of about 43-45% was recorded in untreated control and this was attributed to the damage caused by insect pests of budding, flowering and podding stages. Cowpea treated with Cymbush super EC and NSAE had 16-31 and 31-34% grain loss, respectively. IT98k-131-2 sprayed three times with either Cymbush super EC or NSAE gave consistently the best grain yield in both seasons. However, NSAE gave averagely higher marginal return (25.45) than Cymbush super EC (18.00) in the study. Three sprays also gave the highest marginal returns over control. Insecticide application once each at budding (35-40 DAS), flowering (50%) and podding (10 day after second spray) was effective in reducing insect pests' infestation and increased grain yield of rainfed cowpea in the Lake Chad shore area. Three sprays of either Cymbush super EC or NSAE gave economically the best control of insect pest and the best grain yield of cowpea. The variety IT98k-131-2 can be cultivated for resistance and high yield. Neem seed aqueous extract can be used as an alternative insecticide for safe, cheap and effective control of insect pests in cowpea.**

**Key words:** Cowpea variety, spray regime, NSAE, insect pest, cymbush super EC, Lake Chad shore.

## INTRODUCTION

Cowpea (*Vigna unguiculata*) popularly known as black eye peas or bean is widely grown in the tropics and subtropics. A major food legume in Africa, it is extensively cultivated in the low land tropics of Asia and Latin America. It is traditionally considered as a food legume of the poorest of the poor and is mostly cultivated by small-scale farmers as a subsistence crop (IITA, 1989). Cowpea is widely grown in the Guinea and Sudan savannas of Nigeria with Borno state being the major producer (Kamara et al., 2007). It is also extensively grown around the shores of Lake Chad basin area of Nigeria as a sole crop.

Insect pest damage is the major cause of low grain yield in cowpea around the Lake Chad shore area where the crop is grown in a monocrop. It was reported that the impact of insect pest attack on cowpea is more pronounced when it is grown in a monocrop (Jackai and Singh, 1983). In a preliminary survey conducted, farmers in the area observed grain loss of more than 75% due to insect pest. Similarly, more than 70% or even entire crop failure was recorded due to insect pest alone (Raheja, 1976; Jackai and Daoust, 1986).

To reduce this huge grain loss, farmers indiscriminately spray insecticide and this has been identified as one of the causes of pest outbreak due to the effect of synthetic insecticide on natural enemies. Environmental effects of insecticides have been of great concern recently and there is no information on effective spray schedule and resistance of the common cowpea cultivars in the area to the major insect pests. The establishment of minimum number of sprays required for an effective control of the insect pest of cowpea is as necessary as the control of the pest itself. The objectives of this study are to determine the most resistant cowpea cultivar to insect pests among the three cultivars evaluated in the study, the spray regime that gives an economic control of cowpea pests and the best yield of the crop and the grain yield loss of cowpea due to insect pests.

## MATERIALS AND METHODS

The experiment was conducted at Baga (13° .29" N and 13° 32" E) in 2008 and 2009 raining seasons.

### Sources of planting materials

Seeds of three cowpea varieties, IT98K- 131- 2, was obtained from IITA Kano substation; the other two varieties, Borno brown and Kanannado, were obtained from Borno State Agricultiural Development Programme (BOSADP) office in Maiduguri. Cymbush super EC® [Cypermethrin (30 g) + dimethoate (250 g)] was purchased from a BOSADP accredited agrochemical dealer in Maiduguri. Neem seed aqueous extract (NSAE) was obtained from a laboratory preparation made following the procedure described by Anaso and Lale (2001).

### Experimental design and treatments

An area of 50 X 30 m was cleared of shrub and grasses and burnt before the first rain of the season.The factorial experiment consisted of three cowpea varieties (IT98K- 131- 2, Kanannado and Borno brown) as vertical factor, an untreated control (sprayed with water only) and two insecticides (Cymbush super EC® and NSAE) as horizontal factor and three spraying regimes (one each at budding, flowering, and podding) as sub plot. Each treatment was allocated to a plot of 4 X 4 m with alleys of 0.75 and 1.5 m between plots and replications, respectively. Each treatment was replicated three times. Seeds dressed with Apron plus® at 10 g / 1 kg were sown at the rate of 2 seeds per hole at the spacing of 75 X 50 cm. Each plot had 35 stands arranged in 5 rows of 7 stands each, with 2 plants per stand. Sowing was conducted on 7 July, 2008 and 15 July, 2009. NSAE was applied at the rate of 2.5 kg / 25 L (w/v)/ha, while Cymbush was applied at 280 g a.i / ha using a CP15 Knapsack sprayer.

### Insect sampling and identification

*Megalurothrips sjostedti* were counted from five flowers randomly picked from each stand in the two outer rows of each plot. The Legume pod borer, *Marucavitrata* was counted from flowers and pods of plants in one of the rows that were sampled for thrips assessment. *Anoplocnemis curvipes* and *Mylabris* spp. adult were counted from the two outer rows of each plot using a tally counter. The counts commenced when the insects appeared on the crop and were done on weekly basis from budding until harvest. All insects were identified, at the insect museum of Institute for Agricultural Research, Ahmad Bello University, Zaria.

### Determination of grain yield (kg/ha)

Matured and dried pods from each of the three inner rows of each plot were harvested. The harvest for each plot was shelled, winnowed and the grains weighed and recorded in kg/ha.

### Assessment of grain yield, grain loss and marginal returns

(i) *Grain yield (kg)* = No. of productive plants / ha X no. of pods / plant X no. of seeds / pod X wt. of a normal seed (Raheja, 1976).

(ii) *Grain loss (kg)* = Total no. of plants / ha X no. of damaged and shed pods and flowers due to damage / plant X no. of damaged seeds / pod X wt. of a normal seed (Raheja, 1976).

$$\text{(iii) } \textit{Grain loss (\% )} = \frac{\text{Grain yield loss (kg)}}{\text{Potential yield (kg) that is Grain yield + Grain yield loss}} \times 100$$

*Corresponding author. E-mail: wacklers@yahoo.com. Tel: +2348060584280.

**Table 1.** Percentage Relative abundance of the insect pests associated with rainfed cowpea at Baga in the Lake Chad Basin area of Nigeria in 2008 and 2009.

| Major insect pests | Percentage Relative abundance/4m$^2$ | | |
|---|---|---|---|
| | 2008 | 2009 | Mean |
| *Megalurothrips sjostedti* | 43.8 | 49.9 | 46.8 |
| *Mylabris spp.* | 32.2 | 41.5 | 36.8 |
| *Maruca vitrata* | 16.8 | 3.9 | 10.4 |
| *Anoplocnemi scurvipes* | 7.2 | 4.7 | 6.0 |

$$\text{(iv) Marginal returns} = \frac{\text{Cost (N) of increase in grain yield per additional spray}}{\text{Cost (N) of additional spray / treatment}}$$

(Amatobi, 1995)

It should be noted that: i. cost of cowpea grain at the prevailing market price shortly after the harvest was N100/kg; ii. Cymbush super EC and its application cost N1700/ha. iii. Neem seed aqueous extract and its application cost N850/ha.

$$\text{(v) Pod damage (\%)} = \frac{\text{No. of damaged pods / plant}}{\text{Total no. of pods / plant / treatment}} \text{ X } 100$$

**Data analysis**

Data were square root transformed and subjected to analysis of variance to determine significant differences between treatments and means were separated using LSD test at 5% probability. Analysis was run by statisti x 8.0 software.

## RESULTS

The results in Table 1 show that in both 2008 and 2009 cropping seasons *M. sjostedti* was the highest in abundance followed by *Mylabris* spp. *A. curvipes* was the lowest in abundance.Table 2 shows that in both 2008 and 2009 rainy seasons, Borno brown did not produce flowers. The number of legume pod borer, Blister beetle and grain yield were significantly higher in IT98k-131-2 than in Kanannado in 2008. In 2009, the number of *A. curvipes* was significantly higher in IT98k-131-2 than in Kanannado; however, grain yield was relatively higher in IT98k-131-2 than in Kanannado. For insecticide effect (Table 2), *M. sjostedti*, *Mylabris* spp. and *A. curvipes* and damaged pod were significantly lower in cowpea treated with Cymbush or NSAE than in untreated control in both seasons. However, grain yield was significantly higher in cowpea treated with Cymbush and significantly lower in untreated control than in cowpea treated with NSAE in 2008. In 2009, grain yield loss was significantly lower in cowpea treated with Cymbush and significantly higher in untreated control than in cowpea treated with NSAE. Cowpea sprayed thrice or twice had significantly higher grain yield and significantly lower grain yield loss, number of *A. curvipes* and *Mylabris* spp. than cowpea sprayed once in 2008 (Table 2). In 2009, grain yield was significantly higher and grain yield loss, number of *A. scurvipes* and *Maruca vitrata* were significantly lower in cowpea sprayed thrice than in cowpea sprayed once. The number of *M. sjostedti* and *Mylabris* spp. were significantly lower in cowpea sprayed thrice and significantly higher in cowpea sprayed once than in cowpea sprayed twice in 2009.

Interaction effects of variety and insecticide in 2008 (Table 3) shows that IT98k-131-2 sprayed with Cymbush had significantly lowered the number of *M. sjosted* than NSAE. Similarly, the number of *M. vitrata* was significantly lower in Kanannado and IT98k-131-2 sprayed with Cymbush than IT98k-131-2 sprayed with NSAE and untreated control. *Mylabris* spp. was significantly lower inKanannado sprayed with either Cymbush or NSAE and IT98k-131-2 sprayed with Cymbush than IT98k-131-2 sprayed with NSAE. Grain yield was significantly higher in IT98k-131-2 sprayed with Cymbushthan untreated control. Grain yield loss was significantly lower in Kanannado and IT98k-131-2 treated with either of the insecticides than in untreated control. While sustaining significantly higher infestation, damaged pod and grain yield loss, the lowest grain yield occurred in the untreated controls.

For variety and spraying regime interaction, *Mylabris* spp. was significantly lower in Kanannado sprayed thrice than IT98k-131-2 sprayed twice or once. *A. scurvipes* was significantly higher in IT98k-131-2 sprayed once than the other treatments except Kanannado sprayed once; the lowest number occurred in Kanannado sprayed thrice. Grain yield loss was significantly higher in IT98k-131-2 sprayed once than in IT98k-131-2 or Kanannado sprayed thrice. Grain yield was significantly higher in IT98k-131-2 sprayed twice or thrice than inKanannado sprayed once (Table 3). For insecticide and spraying regime interaction, three sprays of either Cymbush or NSAE had significantly lowered the number of *M. sjostedti, M. vitrata*, *Mylabris* spp. and *A. scurvipes* and pod damage than untreated control. Grain yield was significantly higher in cowpea sprayed thrice or twice with Cymbush than the untreated control.

Results in Table 4 show that for variety and insecticide interaction, *M. sjostedti* and grain yield loss were significantly lower in Kanannado or IT98k-131-2 sprayed

**Table 2.** Effect of Variety, Insecticide and spray Regime on insect pests, damage and grain yield of rainfed cowpea at Baga, Lake Chad Basin area of Nigeria in 2008 and 2009.

| Treatment | No. of Flower thrips/stand | | No. of Maruca larvae/row | | No. of Blister beetle/row | | No. of PSB/row | | Percentage Damaged pod | | Grain yield Loss (%) | | Grain yield (kg/ha) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 |
| Variety (A) | | | | | | | | | | | | | | |
| Kanannado | 1.28 | 2.19 | 1.16 | 1.15 | 1.21 | 2.11 | 1.06 | 1.08 | 4.94 | 5.06 | 8.31 | 8.06 | 18.85(354.13) | 32.72(1069.270) |
| Borno brown | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| IT98k-131-2 | 1.29 | 2.18 | 1.16 | 1.08 | 1.47 | 1.89 | 1.09 | 1.24 | 4.77 | 4.63 | 8.59 | 7.71 | 22.65(512.02) | 35.75(1276.92)* |
| P-value(0.05) | 0.00 | 0.00 | 0.00 | 0.03 | 0.00 | 0.04 | 0.02 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LSD | 0.08 | 0.13 | 0.08 | 0.09 | 0.18 | 0.83 | 0.05 | 0.09 | 0.70 | 0.58 | 1.01 | 0.56 | 2.48 | 3.26 |
| Insecticide (B) | | | | | | | | | | | | | | |
| Cymbush | 1.11 | 1.55 | 1.03 | 1.07 | 1.11 | 1.60 | 1.02 | 1.09 | 2.63 | 2.72 | 5.66 | 4.09 | 18.87(355.12) | 26.01(675.52) |
| NSAE | 1.16 | 1.82 | 1.09 | 1.08 | 1.15 | 1.53 | 1.03 | 1.03 | 2.96 | 3.26 | 5.62 | 5.89 | 15.77(247.76) | 24.38(593.14) |
| Control | 1.31 | 2.01 | 1.19 | 1.08 | 1.42 | 1.87 | 1.09 | 1.19 | 5.13 | 4.72 | 6.63 | 6.78 | 7.85(60.65) | 19.08(362.97) |
| P-value(0.05) | 0.00 | 0.02 | 0.04 | 0.90 | 0.02 | 0.48 | 0.01 | 0.05 | 0.07 | 0.00 | 0.13 | 0.00 | 0.00 | 0.03 |
| LSD | 0.06 | 0.28 | 0.12 | 0.05 | 0.21 | 0.74 | 0.04 | 0.13 | 0.42 | 0.65 | 1.21 | 0.66 | 2.97 | 4.97 |
| Spraying regime (C) | | | | | | | | | | | | | | |
| Regime #1 | 1.21 | 1.88 | 1.12 | 1.12 | 1.33 | 1.81 | 1.08 | 1.16 | 3.76 | 3.55 | 6.37 | 6.11 | 12.23(148.60) | 21.28(451.79) |
| Regime #2 | 1.19 | 1.80 | 1.12 | 1.07 | 1.19 | 1.66 | 1.04 | 1.10 | 3.40 | 3.73 | 5.95 | 5.62 | 14.62(212.74) | 22.33(497.54) |
| Regime #3 | 1.18 | 1.69 | 1.07 | 1.04 | 1.16 | 1.53 | 1.03 | 1.03 | 3.55 | 3.41 | 5.59 | 5.03 | 15.64(243.74) | 25.86(667.53) |
| P-value(0.05) | 0.12 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.10 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 |
| LSD | 0.03 | 0.07 | 0.04 | 0.04 | 0.06 | 0.08 | 0.03 | 0.05 | 0.22 | 0.39 | 0.41 | 0.31 | 1.03 | 2.73 |
| Interaction | | | | | | | | | | | | | | |
| AB | S | S | S | S | S | NS | S | S | S | S | NS | S | S | S |
| AC | S | S | NS | NS | S | S | NS | S | NS | NS | NS | S | S | NS |
| BC | NS | S | NS | NS | S | S | NS | NS | S | NS | NS | S | S | NS |
| ABC | NS | NS | NS | NS | NS | S | NS | NS | NS | NS | NS | S | NS | NS |

*Figures in parenthesis are untransformed. Regime #1 = spray at budding; Regime #2 = spray at budding and flowering; Regime #3 = spray at budding, flowering and podding.Data are square root transformed.$y = \sqrt{x} + 1$. LSD= least significant difference.

with Cymbush and significantly higher in the untreated control than in Kanannado or IT98k-131-2 sprayed with NSAE. *M. vitrata* was significantly lower in cowpea sprayed with Cymbushthan untreated control. *A. scurvipes* was significantly lower in Kanannado or IT98k-131-2 sprayed with NSAE than IT98k-131-2 sprayed with Cymbush. Damaged pod was significantly lower in Kanannado or IT98k-131-2 sprayed with Cymbushthan IT98k-131-2 sprayed with NSAE. Grain yield was significantly higher in Kanannado sprayed with Cymbush than untreated control (Table 4). For variety and spraying regime, *M. vitrata* and grain yield loss significantly lower in IT98k-131-2 sprayed thrice than in IT98k-131-2 and Kanannado sprayed once. *A. scurvipes* was significantly lower in Kanannado sprayed thrice or twice than IT98k-131-2 sprayed twice or once. Grain yield was significantly higher in IT98k-131-2 sprayed thrice than Kanannado sprayed twice or once (Table 4). For insecticide and spraying

**Table 3.** Effect of interaction on Insect pests, Damage and Grain yield of rainfed cowpea at Baga, Lake Chad Basin area of Nigeria in 2008.

| Interaction | No. of flower thrips/stand | No. of Maruca Larvae/row | No. of Blister beetle/row | No. of pod sucking bugs/row | Percentage damaged pods | Grain yield loss (%) | Grain yield (kg/ha) |
|---|---|---|---|---|---|---|---|
| A x B | | | | | | | |
| A1 x B1 | 1.17 | 1.05 | 1.18 | 1.01 | 3.61 | 7.85 | 25.31(639.49) |
| A1 x B2 | 1.20 | 1.11 | 1.05 | 1.04 | 3.97 | 7.69 | 19.79(390.80) |
| A1 x B3 | 1.48 | 1.20 | 1.39 | 1.12 | 7.25 | 9.39 | 11.43(129.71) |
| A2 x B1 | NA | NA | NA | NA | NA | NA | NA |
| A2 x B2 | NA | NA | NA | NA | NA | NA | NA |
| A2 x B3 | NA | NA | NA | NA | NA | NA | NA |
| A3 x B1 | 1.15 | 1.05 | 1.15 | 1.04 | 3.27 | 8.13 | 30.31(917.45) |
| A3 x B2 | 1.16 | 1.16 | 1.39 | 1.06 | 3.90 | 8.16 | 26.52(702.42) |
| A3 x B3 | 1.20 | 1.29 | 1.88 | 1.15 | 7.14 | 9.51 | 11.12(122.72) |
| P-value(0.05) | 0.00 | 0.04 | 0.01 | 0.04 | 0.67 | 0.38 | 0.00 |
| L S D | 0.06 | 0.09 | 0.18 | 0.06 | 0.61 | 0.94 | 3.04 |
| A x C | | | | | | | |
| A1 x C1 | 1.32 | 1.19 | 1.32 | 1.09 | 5.28 | 8.96 | 16.13(259.27) |
| A1 x C2 | 1.22 | 1.17 | 1.18 | 1.05 | 4.63 | 8.23 | 19.86(393.50) |
| A1 x C3 | 1.28 | 1.09 | 1.13 | 1.03 | 4.93 | 7.74 | 20.54(420.89) |
| A2 x C1 | NA | NA | NA | NA | NA | NA | NA |
| A2 x C2 | NA | NA | NA | NA | NA | NA | NA |
| A2 x C3 | NA | NA | NA | NA | NA | NA | NA |
| A3 x C1 | 1.30 | 1.18 | 1.66 | 1.15 | 5.01 | 9.15 | 19.56(381.59) |
| A3 x C2 | 1.31 | 1.20 | 1.41 | 1.06 | 4.59 | 8.61 | 23.00(527.91) |
| A3 x C3 | 1.25 | 1.11 | 1.35 | 1.04 | 4.72 | 8.03 | 25.39(643.86) |
| P-value(0.05) | 0.04 | 0.25 | 0.00 | 0.08 | 0.46 | 0.12 | 0.00 |
| L S D | 0.12 | 0.11 | 0.24 | 0.06 | 1.47 | 1.03 | 6.29 |
| B X C | | | | | | | |
| B1 x C1 | 1.13 | 1.05 | 1.27 | 1.05 | 2.90 | 6.42 | 15.73(246.40) |
| B1 xC2 | 1.09 | 1.05 | 1.07 | 1.00 | 2.23 | 5.55 | 19.47(378.20) |
| B1 x C3 | 1.10 | 1.00 | 1.00 | 1.00 | 2.76 | 5.00 | 21.41(457.47) |
| B2 x C1 | 1.17 | 1.13 | 1.29 | 1.07 | 3.26 | 5.99 | 13.36(177.41) |
| B2 x C2 | 1.18 | 1.13 | 1.09 | 1.03 | 2.85 | 5.72 | 16.23(262.38) |
| B2 x C3 | 1.12 | 1.02 | 1.06 | 1.00 | 2.76 | 5.13 | 17.73(313.35) |
| B3 x C1 | 1.32 | 1.19 | 1.42 | 1.09 | 5.13 | 6.69 | 7.61(56.87) |
| P-value(0.05) | 0.27 | 0.22 | 0.00 | 0.68 | 0.11 | 0.10 | 0.00 |
| L S D | 0.16 | 0.12 | 0.28 | 0.07 | 2.06 | 3.62 | 10.34 |

Figures in parenthesis areuntransformed.Data are square root transformed. $y = \sqrt{x} + 1$. A1= Kanannado; A2= Borno brown; A3= IT98k-131-2; B1= cymbush super EC; B2=NSAE; B3= untreated control; C1= one spray; C2= two sprays; C3= three sprays.LSD= least significant difference.

regime interaction, the number of *M. sjostedti* was significantly lower in cowpea sprayed thrice with Cymbush than in untreated control. *Marucavitrata* was significantly lower in cowpea sprayed thrice with Cymbush or NSAE than in cowpea sprayed once with NSAE but comparable with the other treatments. *Anoplocnemiscurvipes* did not occur in cowpea sprayed thrice with Cymbush or NSAE however, the number was significantly lower in cowpea sprayed twice with NSAE than in cowpea sprayed once with Cymbush and the untreated control. Damaged pod and grain yield loss were significantly lower in cowpea sprayed thrice with Cymbush than in the untreated control. Cowpea sprayed thrice with Cymbush had the highest grain yield, although there were all comparable (Table 4).

The marginal return obtained on each additional spray

**Table 4.** Effect of interaction on Insect pests, Damage and Grain yield of rainfed cowpea at Baga, in the Lake Chad Basin area of Nigeria in 2009.

| Interaction | No. of Flower thrips /stand | No. of Maruca Larvae /row | No. of Blister beetle /row | No. of Pod Sucking Bugs /row | Percentage Damaged pods | Grain yield loss (%) | Grain yield(kg/ha) |
|---|---|---|---|---|---|---|---|
| **A x B** | | | | | | | |
| A1 x B1 | 1.83 | 1.11 | 1.84 | 1.09 | 3.70 | 6.08 | 39.26(1540.66) |
| A1 x B2 | 2.24 | 1.16 | 1.80 | 1.05 | 4.14 | 8.29 | 36.41(1324.69) |
| A1 x B3 | 2.52 | 1.23 | 2.69 | 1.10 | 7.33 | 9.79 | 22.47(503.95) |
| A2 x B1 | NA | NA | NA | NA | NA | NA | NA |
| A2 x B2 | NA | NA | NA | NA | NA | NA | NA |
| A2 x B3 | NA | NA | NA | NA | NA | NA | NA |
| A3 x B1 | 1.81 | 1.10 | 1.97 | 1.17 | 3.45 | 5.21 | 37.77(1425.20) |
| A3 x B2 | 2.22 | 1.14 | 1.79 | 1.05 | 4.64 | 8.37 | 35.72(1274.63) |
| A3 x B3 | 2.51 | 1.20 | 1.91 | 1.49 | 5.82 | 9.53 | 33.76(1139.01) |
| P-value(0.05) | 0.04 | 0.02 | 0.38 | 0.00 | 0.54 | 0.00 | 0.01 |
| L S D | 0.19 | 0.07 | 0.49 | 0.11 | 0.73 | 0.92 | 5.18 |
| A1 x C1 | 2.32 | 1.20 | 2.33 | 1.15 | 4.82 | 8.65 | 29.74(883.41) |
| A1 x C2 | 2.22 | 1.14 | 2.07 | 1.07 | 5.39 | 7.93 | 31.19(971.88) |
| A1 x C3 | 2.05 | 1.10 | 1.94 | 1.03 | 4.95 | 7.59 | 37.22(1383.96) |
| A2 x C1 | NA | NA | NA | NA | NA | NA | NA |
| A2 x C2 | NA | NA | NA | NA | NA | NA | NA |
| A2 x C3 | NA | NA | NA | NA | NA | NA | NA |
| A3 x C1 | 2.31 | 1.14 | 2.12 | 1.34 | 4.83 | 8.69 | 33.09(1094.41) |
| A3 x C2 | 2.19 | 1.08 | 1.90 | 1.24 | 4.80 | 7.93 | 34.79(1209.62) |
| A3 x C3 | 2.04 | 1.03 | 1.65 | 1.13 | 4.27 | 6.49 | 39.35(1547.74) |
| P-value(0.05) | 0.01 | 0.08 | 0.00 | 0.04 | 0.01 | 0.00 | 0.18 |
| L S D | 0.29 | 0.08 | 0.53 | 0.14 | 1.29 | 1.55 | 6.31 |
| **B X C** | | | | | | | |
| B1 x C1 | 1.66 | 1.13 | 1.74 | 1.18 | 2.91 | 5.09 | 23.40(546.61) |
| B1 xC2 | 1.57 | 1.06 | 1.62 | 1.08 | 2.85 | 3.99 | 24.11(580.44) |
| B1 x C3 | 1.41 | 1.03 | 1.45 | 1.00 | 2.38 | 3.21 | 30.52(930.16) |
| B2 x C1 | 1.96 | 1.14 | 1.83 | 1.08 | 3.05 | 6.47 | 21.36(455.12) |
| B2 x C2 | 1.81 | 1.08 | 1.45 | 1.02 | 3.62 | 6.09 | 23.79(565.11) |
| B2 x C3 | 1.69 | 1.03 | 1.32 | 1.00 | 3.10 | 5.11 | 27.98(781.66) |
| B3 x C1 | 2.01 | 1.08 | 1.87 | 1.23 | 4.70 | 6.78 | 19.08(362.97) |
| P-value(0.05) | 0.02 | 0.12 | 0.00 | 0.40 | 0.00 | 0.00 | 0.16 |
| L S D | 0.59 | 0.09 | 0.70 | 0.16 | 2.06 | 3.42 | 16.62 |

Figures in parenthesis are untransformed.Data are square root transformed. $y = \sqrt{x} + 1$. A1= Kanannado; A2= Borno brown; A3= IT98k-131-2; B1= Cymbush super EC; B2= NSAE; B3= untreated control; C1= one spray; C2= two sprays; C3= three sprays.LSD= least significant difference.

of Cymbush or NSAE in both 2008 and 2009, was positive (Table 5). Higher percentage increase in grain yield was recorded in cowpea treated with Cymbush than with NSAE in both 2008 and 2009.

## DISCUSSION

The failure of Borno brown to produce flowers in both 2008 and 2009 rainy season indicates that Borno brown is not suitable for rainy season cultivation in the Lake Chad Basin area. However, this may be due to short duration of rainfall (average of 78 days) experienced in the area over the study period. It was earlier reported that pod set in cowpea could be affected by moisture stress (Ojehomon, 1968; Dzemo et al., 2010). In contrast, Kanannado, also a long duration variety (90-120 days), performed well over the same period. The reason for this

**Table 5.** The marginal returns of rainfed cowpea for different spray regimes of Cymbush and NSAE in 2008 and 2009 cropping seasons.

| Spray level | Cymbush | | | NSAE | | |
|---|---|---|---|---|---|---|
| | Grain yield (kg/ha) | MR | Grainyield increase over control (%) | Grain yield (kg/ha) | MR | Grainyield increase over control (%) |
| **2008** | | | | | | |
| Control | 56.87 | | | 56.87 | | |
| Regime #1 | 246.40 | 11.15 | 333.27 | 177.41 | 14.18 | 211.96 |
| Regime #2 | 378.20 | 18.90 | 565.03 | 262.38 | 24.18 | 361.37 |
| Regime #3 | 457.47 | 23.57 | 704.41 | 313.35 | 30.17 | 451.00 |
| **2009** | | | | | | |
| Control | 362.00 | | | 362.00 | | |
| Regime #1 | 546.61 | 10.86 | 51.00 | 455.12 | 10.96 | 25.72 |
| Regime #2 | 580.44 | 12.85 | 60.34 | 565.11 | 23.90 | 56.11 |
| Regime #3 | 930.33 | 33.42 | 157.00 | 781.66 | 49.37 | 115.93 |

MR= marginal return. Regime #1 = spray at budding; Regime #2 = spray at budding and flowering Regime #3 = spray at budding, flowering and podding.

variety differences not is readily explainable. Although, Kanannado is known to be suitable for dry season cultivation (Singh et al., 1996), suggesting that the variety may be more tolerant to harsh conditions than Borno brown. Significantly higher number of *M. vitrata* and *Mylabris* spp. were accompanied by higher grain yield in IT98k-131-2 than in Kanannado in 2008, suggesting that IT98k-131-2 performed better than Kanannado despite the higher infestation by the insect pests. Moreover, untreated IT98k-131-2 had significantly higher grain yield than untreated Kanannadoin 2009 although these were comparable in 2008. This suggests that IT98k-131-2 may be more tolerant to infestation and damage by insect pests of flowering and podding stages than Kanannado. Kamara *et al*. (2007) and Oniyebe et al. (2006) reported that IT98k-131-2 has profuse flowering and podding ability. It was possible that IT98k-131-2 may have compensated for insect pests damage by producing more flowers and pods.

IT98k-131-2 treated with Cymbush super EC had significantly higher grain yield than Kanannado. However, both insecticides significantly reduced *M. sjostedti*, *A. scurvipes*, damaged pods and significantly increased grain yield than the untreated control in both 2008 and 2009. Nevertheless, the prospect of higher grain yield from profuse flowering and podding in the face of insect pests' damage is likely to be higher with a combination of increasing sprays of Cymbush and IT98-131-2 than with the other combinations of varieties and insecticides.

Significant reduction of insect pests' infestation and grain yield loss and increase in grain yield were achieved by applying insecticide two or three times, once each at budding and flowering or once each at budding, flowering and podding stages compared to when applied once at budding. The result implies that farmers in the Sahel area of the Lake Chad Basin can significantly improve grain yield and reduce grain yield loss from insect pests' infestation and damage by applying two or three sprays of insecticide. Dugje et al. (2009) reported that 2-3 sprays of insecticide are required for a good crop of cowpea in Northern Guinea savanna. In this work, grain yield increased by 8.98, 6.63 and 5.83% in 2008 and 13.31, 1.69 and 7.40% in 2009 for one, two and three sprays, respectively of Cymbushover NSAE compared with the control. Clearly, the increase in grain yield from Cymbush compared with NSAE was larger only for the first spray at budding; the increases were not much for two and three sprays at flowering and podding respectively, over the study period. Farmers will benefit more by using NSAE sprays if more than one spray is required to control the insect pests in cowpea fields.

Two to three sprays of Cymbush was more effective against *M. sjostedti*, *Marucavitrata*, *Mylabris* spp. and *A. scurvipes* than sprays of NSAE; however, three sprays of NSAE significantly lowered the number of *Mylabris* spp. and *A. scurvipes*. Consequently, the number of damaged pod was significantly lowered by 2-3 sprays of either Cymbush or NSAE; however, grain yield was significantly higher with 2-3 sprays of Cymbush. This result implies that farmers in the area can control insect pests of budding, flowering and podding stages with 2-3 sprays of Cymbush or 3 sprays of NSAE or a combination of the two to increase grain yield. The marginal return shows that spraying cowpea up to three times is more profitable than spraying once or twice. However, relatively higher marginal returns were recorded with NSAE than with Cymbushin this study. This may have been due partly to the differences in the cost of the pesticides. This result implies that NSAE could be used as an alternative to or in combination with synthetic insecticide to control insect

pests for a profitable cowpea production. Egho (2011) reported that neem bio-pesticide can form a component of an Integrated Pest Management Programme of cowpea pest.

The percentage relative abundance of insect pests of cowpea in the area showed that *M. sjostedti*, *Mylabris* spp., *M. vitrata*, and *A. curvipes* in descending order were the major insect pests encountered during the study period. It was reported earlier that *M. sjostedti*, *M. vitrata* and *A. curvipes* are the most important insect pests of cowpea in Nigeria (Amatobi, 1995; Kyamanyawa, 1996; Karungi et al., 2000; Dzemo et al., 2010).

## Conclusion

It can be concluded that, IT98k-131-2 has some degree of resistance to insect pests of budding, flowering and podding stages when compared to Kanannado. Pod damage and grain loss were reduced by application of Cymbush and NSAE. However, Cymbush was more effective than NSAE. The spraying regime for the best and economic grain yield of cowpea can be achieved by three sprays of either Cymbush or NSAE applied once each at budding, flowering and podding stages. Consequently, the marginal return on the use of NSAE appeared to be more advantageous. Alternatively, Cymbush can be used at a highly reduced rate when integrated with NSAE, thereby reducing the risk of exposure and damage these might cause the sole user of synthetic Cymbush. The major insect pests of cowpea in the study area are *M. sjostedti*, *Marucavitrata*, *Mylabris* spp. and *A. scurvipes*.

## Recommendations

It is recommended that the variety IT98k-131-2 be cultivated for high yield and resistance to some major insect pest of cowpea. Also, Neem Seed Aqeuous Extract is a cheap, safe, and effective bio insecticide for the control of insect pest of cowpea.

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