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Management of the legume pod borer *Maruca vitrata* Fabricius (Lepidoptera: Crambidae) with field applications of the entomopathogenic fungus, *Beauveria bassiana* and a mixed formulation of the baculovirus *Mavi*MNPV with emulsifiable neem oil

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The present study assessed the effectiveness of the entomopathogen fungus *Beauveria bassiana* in comparison with neem oil combined with the baculovirus *Mavi*MNPV and a synthetic insecticide deltamethrin for the control of *Maruca vitrata* Fabricius under field conditions. The trial was conducted in six villages at Glazoue and Djakotomey, two districts in Benin. Four treatments consisting of (1) untreated control, (2) neem + *Mavi*MNPV, (3) *B. bassiana* and (4) deltamethrin were arranged in a complete randomized block design with three replicates. Reproductive organs of cowpea plants were sampled for estimating population of *M. vitrata*. In spite of the importance between-field variations observed, bio-pesticides significantly reduced the population density of *M. vitrata* as well as its damage level. Similarly, grain yields were improved in sprayed cowpea compared to the untreated control, regardless of the experimental zone. In Glazoue, yields ranged from 724.06 ± 5.04 kg/ha (deltamethrin) to 933.03 ± 8.7 kg/ha (*B. bassiana*). In Djakotomey, 649.1±4.7, 611.07±5.1, 583.19±4.04 and 217.11±3.9 kg/ha were recorded for deltamethrin, *B. bassiana*, neem + baculovirus, and untreated control, respectively. The possibilities of the using bio-pesticides as efficient IPM components in cowpea are discussed.

Key words: Cowpea pests, bio-pesticides, Beauveria bassiana, neem, baculovirus MaviMNPV, deltamethrin.

INTRODUCTION

Cowpea Vigna unguiculata L. (Walp) is one of the most important grain legumes in tropical and sub-tropical

regions of the world (Singh et al., 2002). It is also a multipurpose crop grown as food and feed. Cowpea is

cultivated worldwide with an estimated annual production of 5.4 million tons with Africa producing nearly 5.2 million (FAOSTAT, 2015). Cowpea provides the cheapest source of protein for human consumption (Ajeigbe and Singh, 2006). Cowpea also is rich in vitamins and minerals. Its leaves are also consumed as fresh vegetables, while the plant after harvest is a valuable source of fodder for cattle. The plant tolerates drought, performs well in a wide variety of soils, and being a legume, it replenishes low fertility soils through deposited organic matter. Also, legumes play a key role in soil fertility improvement through biological nitrogen fixation (Ajeigbe and Singh, 2006). Cowpea is grown mainly by small-scale farmers in developing regions.

In Benin, cowpea is the most cultivated and consumed grain legume. In 2014, about 93488 tons of cowpeas were produced in Benin on 115,000 ha (FAOSTAT, 2015). It plays a role in human nutrition especially in rural areas characterized by unbalanced diet. However, the production is low and only 6.12 Kg of cowpea grain were supplied per capaiata per year (FAOSTAT, 2015). Indeed, various constraints seriously limit cowpea production. Of these, biotic constraints namely damage by insect pests remain the most important affecting both yield and quality of the harvested products (Egho, 2010). Of these, the legume pod borer, Maruca vitrata Fabricius (Lepidoptera, Crambidae) is reported to cause serious damage on cowpea. The incidence of larvae of the pest is all the more serious as each larvae attacks several organs of the same plant: Flowers, leaves, flower buds and green pods before migrating to another plant (Liao and Liu, 2000). The caterpillars destroy both cowpea flowers and pods causing yield losses up to 80% (Tamò et al., 2003). Indeed, its larvae, brown, yellow or light green at hatching, darken as they pass from one stage to another. They are hairy, blackheaded with dark brown spots on the body (Datinon, 2005). Several methods have been developed or tested against M. vitrata with chemical pesticides being the most used control means (Jackai, 1995). Chemicals application is no more attractive considering the so many side effects including environmental pollution and human health hazards, pest resistance and resurgence, secondary pest outbreaks and loss of biodiversity (Ekesi et al., 2002; Atachi et al., 2007). Alternative control methods such as host plant resistance and cultural control practices were not much effective to keep insect pests populations below economic thresholds. Investigation on cowpea selection did not yield in lines demonstrating a satisfactory level of resistance to the pod. Only Vigna vexillata (L.) A. Rich, a wild Vigna was identified as highly resistant to M. vitrata. Although V. vexillata is

close to cowpea, it was not possible to transfer the desirable genes into cultivated cowpea varieties because of a strong cross-incompatibility at both pre and postfertilization levels. Also, various cultural practices have been tested but required additional chemical application to achieve efficient control (Oso and Falade, 2010). Hence, the only alternative to overcome damage by the cowpea pod borer *M. vitrata* remains biological control. It consists of using various living organisms such as predators, parasitoids and entomopathogens to control pests while maintaining biodiversity and reducing production costs (Scholz et al., 1998; Girling, 1992). Among the entomopathogenic organisms identified for the virus Maruca vitrata. vitrata multiple М nucleopolyhedrovirus (MaviMNPV) was highly specific to M. vitrata (Lee et al., 2007). It was isolated from dead M. vitrata larvae in Taiwan, and was tested with success in West Africa against M. vitrata (Lee et al., 2007; Tamo et al., 2010; Sokame et al., 2015; Muhammad et al., 2017). Likewise the entomopathogenic fungi such Beauveria bassiana (Balsamo) Vuillemin as (Hypocréales: Ophiocordycipitacea) are known for their ability to infect a wide range of insects including M. vitrata (Srinivasan et al., 2014). In fact, the Beauveria genus is mainly based on the special mode of conidial formation. Phialides carrying conidia are elongated sometimes spherical (Tanada and Kaya, 1993). In Africa and particularly in Benin, B. bassiana has been used to infect a wide range of insects in the field (Godonou et al., 2009; Douro Kpindou et al., 2011). Preliminary studies conducted in the laboratories of the International Institute of Tropical Agriculture (IITA-Benin) on the third and fourth instars of *M*. vitrata using the entomopathogenic fungi, *B. bassiana* (isolate Bb115) have shown that they are effective at various doses on the pest (Douro Kpindou et al., 2011; Toffa Mehinto et al., 2014a). The effect of *B. bassiana* was compared with that of Decis, a chemical pesticide commonly used in cowpea production in Benin and Neem + Virus already used in the biological control of cowpea pests (Sokame et al., 2015).

This study aims to assess the effectiveness of the entomopathogen fungus *B. bassiana* in comparison with the combination of neem oil and the virus *Mavi*MNPV as alternative to synthetic chemicals in the context of pests' management in cowpea.

MATERIAL AND METHODS

Experimental sites

The works described below were conducted at the laboratory

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of insect pathology of the International Institute of Tropical Agriculture (IITA), Benin Station (6°28N and 2°21E, 15 m altitude), near Cotonou, Benin, in climate chamber with a mean temperature of $26 \pm 0.50^{\circ}$ C and a relative humidity of $65.5 \pm 5\%$.

The experiments were conducted also in two agro ecological zones of Central (Glazoue/Ouedeme) and Southern Benin (Djakotomey/Djakotomey 1). Sites that hosted the experiments were used to grow cowpea with different cropping systems. The climate at both sites is of Sudano-Guinean or subequatorial type, with two dry seasons alternating with two rainy seasons. The average rainfall ranged between 700 and 1100 mm per year at Djakotomey with temperature of 23 to 31.2°C. But at Glazoue, the annual rainfall varies between 960 and 1256 mm and the mean temperatures between 24 and 29°C (Capo-Chichi and Guidibi, 2006).

Plant material

The cowpea variety "Wankoun" and "Sakaouga", were used in the different experiments in the agro ecological zones of Central (Glazoue) and southern Benin (Djakotomey 1), respectively. The variety "Wankoun" is the common cultivated one at Galazoue while "Sakaouga" is the dominant variety at Djakotomey. Both varieties are semi-erect varieties with a development cycle lasting for 65 to 70 days.

Bio-pesticides and synthetic pesticide

The control agents used were the virus Maruca vitrata Multiple nucleopolyhedrovirus (MaviMNPV) combined with the botanic pesticide "TopBio" (with neem oil (azadirachtin). The "TopBio" as main component and trace essential oil such as citronellal, citronellol, geraniol and nimbin from lemongrass); Topbio was purchased at BioPhyto-Collines, an artisanal plant extract factory in Benin. Also, the biopesticide of the entomopathogenic fungus B. bassiana isolate (Bb 115) and the chemical pesticide Deltamethrin, Decis (12.5 EC) was used. Colonies of B. bassiana was mass produced from dried conidia in a stock culture at IITA Benin. Conidia viability after 24 h incubation on Potato Dextrose Agar (PDA) was 95%. Chemical pesticide included in the present study was that applied by producers in the study area. The virus MaviMNPV, a baculovirus was obtained from a stock culture at IITA Benin. The virus was introduced in Benin through a collaborative research between IITA-Benin and "World Vegetable Center (WorldVeg)", in Taiwan. The virus was isolated from infected larvae of M. vitrata on "Sesbania Pea (Sesbania cannabina)" in Taiwan (Lee et al., 2007, Chen et al., 2008).

Indeed, authors such as Gouissi (2013) and Sokame et al. (2015) found that the formulation Neem + virus is more effective on *M. vitrata* than the two products used individually.

Experimental set up

Experiments were set up at the two experimental site:

1. In three villages of Glazoue/Ouedeme: Dogbo (08° 00 786' N, 002° 08 198'E, 591m Alt), Gbaglavigo (08° 02 558'N, 002° 09 190'E, 682m Alt) and Allenoudji (08° 01 406'N, 002° 10 760'E, 674 m Alt) where the villages were considered as replications. 2. In three villages of Djakotomey/Djakotomey 1: Bitouhoue (N 06° 53 649'N, 001° 41 689'E, 153 m Altitude), Atchouhoue (06° 52 507' N, 001° 41 919'E, 137m Altitude) and Hounkemey (06° 52 357' N, 001° 41 142' E, 142 m Altitude) where the villages were considered as repetitions.

Farmers' fields

Six cowpea producers were selected from each of six submunicipalities of Glazoue (Ouedeme) and Diakotomey (Djakotomey 1). The field experiments consisted of four treatments: Untreated control (no pesticide), cowpea treated with Topbio (neem) + virus (MaviMNPV), cowpea treated with Deltamethrin and cowpea treated with B. bassiana (isolate Bb 115). Each treatment was repeated three times in a randomized complete block design (RCB) with experimental units of 100 m² (10 m x 10 m). Cowpea was sown at 75 cm x 25 cm. Plants were weeded twice. Maize was planted in alleys to avoid interference between treatments. Biopesticide was applied at a rate of 75 g conidia (active ingredient)/ha which corresponds to a quantity of 0.78×10^{13} conidia/ha and the application volume of biopesticides was two litres per hectare. The dose of MaviMNPV virus used was 1.6×10^{11} OB of virus which is combined against one litre of neem (Topbio). The recommended application rate used in chemical pesticides plots was one litre per hectare for Decis. Plants were treated at the 32^{nd} , 39^{th} , 43^{th} , 49^{st} and 30^{th} , 37^{th} , 41^{st} and 47th days after planting (DAP) in Glazoue and Djakotomey, respectively. The different treatment dates are chosen according to the vegetative cycle of each cowpea variety used in each study area. Plant treatment started at the onset of flowering (flower buds stages), a period of *M. vitrata* infestation. All applications were done using a sprayer SWISSMEX-8l". Temperatures and the mean relative humidity (± SE) recorded during applications were as follows: Tmin = 25.2 ± 0.2°C; Tmax = 31.7 ± 0.5°C; RHmin = 63.3 ± 3.1% and RHmax = 93.9 ± 2.1%.

Assessment of *M. vitrata* population density

Samples of 10 plants were weekly selected randomly per treatment to assess *M. vitrata* population. Flowers and pods were collected from each plant and stored in boxes containing 65% alcohol before treatment (Gouissi, 2013). So, *M. vitrata* were identified and dead or alive larvae were counted four days after treatment. Larvae were put individually in a 3.8 cm \times 2.9 cm \times 4.0 cm boxes with perforated cover for aeration. Cadavers were collected daily. They were put in Petri dishes (Ø = 9 cm) for 24 h to be dried out, and incubated in Petri dishes containing wet filter paper. The number of alive and dead larvae was recorded.

Assessment of damage on the reproductive organs (flowers and pods)

This parameter was determined weekly from the rows. Damage of reproductive organs (organs stung, rotten) was assessed weekly. The presence of *M. vitrata* was checked four times (42nd DAP for flowers and 48th DAP for pods). Thus, ten flowers and/or 10 pods were randomly sampled per treatment.

Holes on flowers or pods and presence of *M. vitrata* larvae were recorded to estimate its damage index.

Weather conditions

The environment factors such as rainfall, temperature and relative humidity were recorded during the experiments. The rainfall was well distributed with minor variations. The months of May and June were the wettest with 108.2 and 94.2 mm, respectively in the central zone (Glazoue) and 192.2 and 101.7 mm, respectively in the southern zone (Djakotomey). In Glazoue, the maximum temperature averaging $28.9 \pm 3.2^{\circ}$ C was recorded in April (beginning of the experiments). It has decreased in July, the period of cowpea maturity.

Cowpea yield assessment

Yield was estimated at 65-70 days after planting (pods maturity period) by harvesting cowpea in three delimited areas (quadrants) similar to those used for *M. vitrata* population estimation. Quadrants consisted of 1 m² with 10 cowpea plants. Pods harvested on the 10 plants were kept in polyethylene bags and labelled according to treatments. Pods were dried by means of sunlight for 3 days and then shelled with hands. Grains in each treatment were weighed with triple beam balance (Mettler PJ 300).

Statistical analysis

Effects of synthetic pesticide (Decis), neem, *Mavi*MNPV virus and *Beauveria bassiana* suspension on the population and damage (%) by *M. vitrata* on different reproductive organs were compared by performing ANOVA using SAS software followed by the test of Student-Newman-Keuls. Percent data (larval mortality rate, sporulation rate of dead larvae) in the different treatments were log-transformed [log (x +1)] prior to the analysis.

RESULTS

Effect of *B. bassiana,* neem oil combined with MaviMNPV on *M. vitrata* population

At Glazoue, the different products namely *B. bassiana*, Decis and neem+MaviMNPV significantly reduced *M. vitrata* larval density compared to untreated control (*F*=10.61; *P* < 0.0001). Indeed, shortly after the first application the average number of *M. vitrata* larvae was lower (0.13±0.6 larvae/plant) in plants treated with Deltamethrin than those treated with biopesticides. Then after the third application, this number slightly increased in Decis treatment (0.9±0.4 larvae/plant) at 46 days after planting (DAP). From this same DAP (46 DAP), the mean number recorded in biopesticide treatments decreased progressively. Despite their slow action, the bio products particularly *B. bassiana* significantly reduced the larval population over the period of this trial (Figure 1A).

At Djakotomey, the different products tested showed comparable efficacy over time. However, from 42 DAP, the number of *M. vitrata* larvae significantly increased for all tested products and reached at the end of the experiments, 1.5 ± 0.04 ; 2.3 ± 0.01 and 2.5 ± 0.02 larvae for Deltamethrin, *B. bassiana* and Neem + *Mavi*MNPV, respectively at 53 DAP (Figure 1B). The curve describing the fluctuation of *M. vitrata* larvae in untreated control was above those that describe the fluctuation in treated cowpea (Figure 1B).

In contrast to Glazoue (Ouedeme), the average number of *M. vitrata* larvae did not decrease at 46 DAP but showed a constant trend till the end of the experiment. However, significant differences were observed between the different products applied (F=2.31; P = 0. 1361).

There were significant interactions between the different pesticides used and the agro-ecological zones pointing at a sharp link between the number of alive *M*.

vitrata larvae and the agro-ecological zone (F= 1.78; P = 0.0363).

Effect of synthetic chemical and bio-pesticides on *M. vitrata* population and damage to fruiting organs in cowpea producers' fields

At Glazoue, for all pesticides used, the damage was heavier on the pods than on the flower. These ranged from 9.2±3.5% (Neem+*Mavi*MNPV, flower buds) to 13.7±2.1% (Neem+*Mavi*MNPV, pods) (Table 1). However, statistical analysis reveals significant differences between treatment for the percent damage in the reproductive organs and controls at Glazoue (F = 13.16, P = 0.0018; F = 2.84, P = 0.0051 for flowers and pods, respectively).

The presence of *M. vitrata* in reproductive organs was highly variable (Table 1). The average number of *M. vitrata* ranged from 0.08 \pm 0.02 (Deltamethrin) to 0.97 \pm 0.08/plant (Neem+*Mavi*MNPV) for flowers and 0.02 \pm 0.01 (Deltamethrin) to 0.18 \pm 0.3/plant (Neem+*Mavi*MNPV) for the pods. Pesticides have better controlled the pest. Significant differences were observed between the different products applied and untreated control for the average number of *M. vitrata* larvae recorded on flowers and pods (Table 1).

At Djakotomey, the number of *M. vitrata* larvae recorded on flowers was lower in Decis-treated plants compared to untreated control. Furthermore, statistical analysis reveals significant differences between treatment for the percent damage in the reproductive organs and controls (F=6.38, P=0.0024; F=2.37, P=0.0374 for flowers and pods respectively) (Table 1). Thus, compared to untreated control, *B. bassiana* significantly reduced the damage level in pods while the combination of neem with *Mavi*MNPV treatment significantly reduced the damage to flowers (Table 1).

Moreover, the monitoring and incubation of dead larvae collected from field revealed that only larvae collected from plots treated with the fungus *B. bassiana* have sporulated.

Yields in cowpea producers' field

The overall grain yield was improved in treated cowpea compared to the untreated control, regardless of the experimental zone (Figure 2). At Glazoue, yields ranged from 858.1 \pm 5.04 kg/ha (Deltamethrin) to 1012.03 \pm 8.7 kg/ha (*B. bassiana*). The performance of *B. bassiana* was significantly better than those of other treatments (*F* = 13.62, *P* = 0.0002). Unlike at Glazoue, yields ranged from 217.2 \pm 3.04 Kg/ha (untreated control) to 649.7 \pm 5.2 Kg/ha (Deltamethrin) at Djakotomey (Figure 2). So, the performance of Deltamethrin was better than those of other treatments. However, all treatments had significantly similar yields.



Figure 1. Fluctuation of *M. vitrata* population density under the influence of chemical and biological pesticides in central (A: Glazoue) and southern (B: Djakotomey) zones at Benin.

DISCUSSION

Both the biopesticides and synthetic pesticide used in this study have significantly reduced the population of *M. vitrata* compared to untreated control. So, during the late

cropping season, all the major insect pests were encountered in the study areas - an observation confirming, that reported by Jackai and Raulston (1988) according to which the major cowpea insect pests occur wherever the crop is cultivated. **Table 1.** Effect of the synthetic pesticide (Decis (deltamethrin)), the combined formulation of a neem-based botanical pesticide Topbio with the baculovirus *Mavi*MNPV, and the formulation of the entomopatogenic fungus *Beauveria bassiana* on the population and damage (%) by the legume pod borer *Maruca vitrata* on different cowpea reproductive structures in central (Glazoue) and southern (Djakotomey) zones at Benin.

Zone	Treatment	Flower		Pods	
		**Percent damage	* M. vitrata larvae	**Percent damage	* M. vitrata larvae
Central zone	Untreated control	66.5±3.7 ^a	1.73±0.4 ^a	17.2±4.8 ^a	1.94±0.04 ^a
	Decis	12.±2.4 ^b	0.08±0.02c	11.8±3.6 ^b	0.01 ^b
	Neem+	9.2±3.5 ^b	0.97 ± 0.08^{b}	13.7±2.1 ^b	0.18±0.3 ^b
	B. bassiana	11.2±4.1 ^b	0.12±0.02 ^b c	8.3±2.7 ^b	0.02 ^b
Southern zone	Untreated control	69.5±6.1 ^a	3.04±0.6 ^a	33.5± 2.1 ^a	3.82±1.0 ^a
	Decis	12.6±2.2 ^b	0.16±0.07c	11.8±1.1 ^b	1.44±0.1 ^b
	Neem+	13.5±1.4 ^b	0.32±0.1 ^b	14.9±1.9 ^{ab}	2.11±0.7 ^{ab}
	B. bassiana	14.9±3.5 ^b	0.31±0.1 ^b	8.9±1.7 ^b	2.07 ± 0.9^{ab}

*Average number per plots \pm SE (calculated on the basis of 3 replicates of 4 observations and on 10 organs); ** Average rates of flowers or damaged pods \pm SE (calculated on the basis of 3 replicates of 4 observations and on 10 organs); Mv = *M. vitrata;* Means in the same column followed by the same letter are not significantly different at the 5% level (ANOVA followed by SNK).



Figure 2. Cowpea yields (kg/ha) in central (Glazoue) and southern (Djakotomey) zones in Benin.

The degree of pest control results in a damage reduction on reproductive organs (flowers and pods). In addition, the synthetic insecticide rapidly reduced larval density than did bio-insecticides. The occurrence and distribution of insect species in this study in the two locations (central and southern zones) followed different trends. So, the chemical pesticide had reduced more rapidly larval density than the biopesticides, due to its broadspectrum action. This insecticide protected old and new leaves including those that were not present during the application. Under the conditions of proper spraying, larval mortality was observed 2 days after application. These results confirm studies by Atachi and Sourokou (1989) and Bognaho (1996) pointing at the effectiveness of Deltamethrin (Decis) on larval stages of cowpea pest. This can probably be explained by resistance of *M. vitrata* to Deltamethrin. Indeed, Ekesi (1999) reported the resistance to several chemical compounds including pyrethroids. However, this needs to be established during future investigations in Benin.

The dose and application volume should adjust to get similar results with the synthetic pesticide for large cowpea plots. The application of *B. bassiana* was slightly more effective than the combination Neem+*Mavi*MNPV. Indeed, the positive action of *B. bassiana* 115 was confirmed by the sporulation observed in the dead larvae collected in fungus *B. bassiana* treated plots. Similar results were reported by Adanvè (2012) and Toffa Mehinto et al. (2014a, 2014b) who confirmed the pathogenicity and virulence of *B. bassiana* 115 to control *M. vitrata* in the laboratory with infection rates ranging from 43 to 98% between 4 to 10 days after treatment). Tumuhaise et al. (2015) also confirmed the effectiveness of both *Metarhizium anisopliae* and *Beauveria bassiana* against *M. vitrata*.

Several authors reported that the genus Beauveria attacks a variety of insects (Prior, 1992). The limited action of the combination Neem+MaviMNPV was not consistent with the observation of Gouissi (2013) who reported that Neem+MaviMNPV were effective against M. vitrata. This effectiveness was attributed to the repelling properties of azadirachtin, the active matter of Neem and the specificity of MaviMNPV to M. vitrata (Honfoga, 2007). Similarly, Gahukar (1988) observed that the effect of the extract of Neem kernels is comparable to treatments with deltamethrin in the control of H. armigera's larvae on groundnut plants. Sokame et al. (2015) found that the formulation Neem + MaviMNPV was more effective.

These differences could be explained not only by specificity in the entomopathogen species but also by the variation in environmental conditions of the study areas. Even both entomopathogenic diseases could be spread through dead larvae, the viral disease is specific to M. vitrata larvae, while that of B. bassiana would affect any insect species through sporulation of dead larvae. The viral disease may result from viral particles multiplication in infected larvae (Lee et al., 2007), while the fungus B. bassiana was reported to affect insects through active body entering (physical deterioration from germinating conidia) and physiological alteration from synthesized enzymes (Liu et al., 2003; Cho et al., 2006). Virulence of B. bassiana was reported to depend on its physiological characteristic and enzyme production (Liu et al., 2003). Enzymatic and mechanical pressure enables B. bassiana to destroy host cuticle proteins. However, the fungus also synthetizes toxic nonenzymatic compounds such as beauvericin and basianolide that speed the infestation process (Cito et al., 2016). Furthermore, other traits such as conidial viability, germination speed, hyphal growth rate and pathogenicity are influenced by environmental factors namely temperature and relative humididy and thereby the fungal efficacy (Liu et al., 2003). The agro-climatic conditions including rainfall would also explain differences in insects' activity at Glazoue (Central zone) and Djakotomey (southern zone) especially during the major cropping season. Differences between the two agroecological zones for the percent damage caused to the reproductive organs could also be explained by climatic factors such as the rainfall. Indeed, in the zone southern, high amount of rainfall was recorded, 48 h after spraying reducing then the efficacy of the bio-pesticides. Furthermore, yields were significantly higher in treated plots compared to the untreated ones at both Glazoue and Djakotomey. These results reflected the infestation and damage rates recorded at the two locations during the period of time covered by the present study. Similar observation has been done by Douro et al. (2013) who demonstrated the effectiveness of entomopathogenic fungi M. anisopliae and B. bassiana and Neem oil in the integrated management of *Helicoverva armigera* (Hübner) (Lepidoptera: Noctuidae) on cotton. In the absence of control measures, high losses were recorded (Atachi et al., 2007; Djiéto-Lordon et al., 2007). The current yield levels were a bit lower compared to that obtained (1.036 kg.ha⁻¹) by Affokpon et al. (2013). This is probably due to soil fertility depletion that characterized the experimental sites. Indeed, the sites were used for several years of cowpea monocropping, system in which the overall damage should be high on reproductive organs. A diagnosis of a mineral earth bar in southern Benin cowpea deficiency was also noted by Amadji and Glitho (2005). So, Balogoun (2012) confirms that the decline in soil fertility is a major problem and a major constraint to agricultural production in Benin. Moreover, Producers claimed higher insect infestations during the great cowpea season in southern zone.

Conclusion

This study assessed the efficacy of entomopathogenic fungi *B. bassiana* (isolate Bb 115) and Neem + MaviMNPV on *M. vitrata*. Although the great variation in their effects, the biopesticides tested have significantly reduced the population density of *M. vitrata* as well as the damage level. The overall grain yield was improved in treated cowpea compared to the untreated control, regardless of the experimental zone. The current study revealed that biopesticides could be formulated from *B. bassiana* or neem + MaviMNPV as efficient alternative to synthetic insecticides for the management of major insect pests in cowpea.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

- Adanvè F (2012). Etude comparative des différentes concentrations d'inoculation du champignon entomopathogène *Beauveria bassiana* (Balsamo) Vuillemin dans le contrôle des larves de *Maruca vitrata* (Lépidoptera : Pyralidae), Mémoire pour l'obtention du diplôme de Licence Professionnelle, ENSTA/UAC 73 p.
- Affokpon A, Djènontin JAP, Zoffoun AG, Allagbé MC, Akondé TP, Aïhou K, Kpagbin G, Gotoechan-Hodonou H, Détongnon J, Mensah GA (2013). Effets des variétés de niébé à buts multiples comme précédent cultural sur le rendement du maïs cultivé sur terres de barre dégradées au Sud-Bénin. Bulletin de la Recherche Agronomique du Bénin, Numéro spécial Fertilité du maïs Janvier 2013, ISSN 1840-7099. pp. 58-68.
- Ajeigbe HA, Singh BB (2006). Integrated pest management in cowpea: Effect of time and frequency of insecticide application on productivity. Crop Protection 25:980-925.
- Amadji GL, Glitho M (2005). Diagnostic des carences minérales d'une terre de barre du sud Benin sous le niébé (*Vigna unguiculata*). Annales des Sciences Agronomiques du Bénin 7:69-89.
- Atachi P, Sourokou B (1989). Use of decis and systoate for the control of *Maruca testulalis* (Geyer) in cowpea. Insect Science Application 3:373-381.
- Atachi P, Dannon EA, Rurema DG (2007). Trap cropping and intercropping of pigeon pea (*Cajanus cajan* Millsp.) in pest management of cowpea (*Vigna unguiculata*) in southern Benin: competing risk and pest status in pod attack. Annales des Sciences Agronomiques du Bénin 9:1-20.
- Balogoun I (2012). Essais de validation des formules d'engrais et des périodes de semis recommandées par le modèle DSSAT pour la production de maïs (*Zea mays* L.) au Sud et Centre Benin. Mémoire de Diplôme d'Etudes Approfondies, Faculté des Sciences Agronomiques, Université d'Abomey-Calavi, Benin 78 p.
- Bognaho MDC (1996). Etude comparée d'efficacité de dipel 2X (*B. thuringiensisi*) et de Decis (deltaméthrine) sur les différents stades larvaires de *Maruca testulalis* (GEYER) (Lépidoptère: Pyralidae) en conditions de laboratoire, Mémoire d'Ingénieur, FSA/UAC 109 p.
- Capo-Chichi YJ, Guidibi E (2006). Monographie de la commune de Glazoue. Rapport d'étude, Programme d'Appui au démarrage des Communes 45 p.
- Chen Y, Wu CY, Lee ST, Lo CF, Tsai MF, Wang CH (2008). Genomic and host range studies of *Maruca vitrata* nucleoplyhedrovirus. Journal of General Virology 89:2315-2330.
- Cho EM, Liu L, Farmeire W, Keyhani NO (2006). EST analysis of cDNA libraries from the entomopathogenic fungus *Beauveria* (*Cordyceps*) bassiana. I. Evidence for stage-specific gene expression in aerial conidia, in vitre blastospores and submerged conidia. Microbiology 152:843-2854.
- Cito A, Barzanti GP, Strangi A, Francardi V, Zanfin A, Dreassi E. 2016. Cuticle-degrading proteases and toxins as virulence markers of *Beauveria bassiana* (Balsamo) Vuillemin. Journal of Basic Microbiology 56:941-948.
- Datinon BD (2005). Effet de Bacillus thuringiensis Berliner sur Maruca vitrata Fabricius (Lepidoptera : Pyralidae) ravageur du niébé, Vigna unguiculata (L.) Walp. et son ennemi naturel Phanerotoma leucobasis Kriechbaumer (Hymenoptera: Braconidae). Mémoire de Diplôme d'Etudes Approfondies (DEA), Faculté des Lettres, Arts et Sciences Humaines (FLASH), Université Nationale du Bénin 120 p.
- Djiéto-Lordon C, Aléné DC, Reboul JL (2007). Contribution à la connaissance des insectes associés aux cultures maraîchères dans les environs de Yaoundé – Cameroun. Journal Biological Science

15:1-13.

- Douro Kpindou OK, Djegui DA, Glitho IA, Tamo M (2011). Comparative Study of the Efficacy of Entomopathogenic Fungi, Chemicals and Botanical Pesticides in the Management of Cotton Pests and Their Natural Enemies in Benin. International Journal of Science and Advanced Technology 3:21-33.
- Egho EO (2010). Comparative studies on insect species of cowpea (*Vigna unguiculata*) (L) Walp in two agro- ecological zones during the early cropping season, in delta State, southern Nigeria. Agriculture and biology Journal of North America 1:946-949.
- Ekesi S, Adamu RS, Maniania NK (2002). Ovicidal activity of entomopathogenic hyphomycetes to the legume pod borer. *Maruca vitrata* and the pod sucking bug *Clavigralla tomentosicollis*. Crop Protection 21:589-595.
- FAOSTAT (2015). Agricultural production, crop primary database. Food and Agriculture Organization of United Nation, Rome. Site internet: http://www.fao.org/statistic
- Gahukar RT (1988). Use of neem (*Azadirachta indica*) extracts in the control of insects pests of groundnut. Insect Science and its Application 5:639-640.
- Girling DJ (1992). Principes et application de la lutte biologique in Manuel de lutte biologique; Organisation des Nations Unies pour l'alimentation et l'agriculture 217 p.
- Godonou I, James B, Atcha-Ahowe C, Vodouhe S, Kooyman C, Ahanchede A, Korie S (2009). Potential of *Beauveria bassiana* and *Metarhizium anisopliae* isolates from Benin to control *Plutella xylostella* L. (Lepidoptera: Plutellidae). Crop Protection 28:220-224.
- Gouissi MTM (2013). Production du Baculovirus entomopathologène de *Maruca Vitrata Mavi*MNPV sur substrat naturel en utilisant les graines germées de différentes variétés de niébé. Mémoire pour l'obtention du diplôme d'Ingénieur des Travaux en Production Végétale: Faculté d'Agronomie de Parakou, Bénin 51 p.
- Honfoga AFYJ (2007). Etude expérimentale de la production du biopesticide Mavi NPV et de son efficacité au champ sur Maruca vitrata Fabricius (Lepidoptera: Pyralidae), Foreur des fleurs et gousses du niébé, Mémoire pour l'obtention du diplôme d'ingénieur des travaux, EPAC/UAC 73 p.
- Jackai LEN, Raulston JR (1988). Rearing the legume pod borer Maruca testulalis (Geyer) (lepidoptera: Pyralidae) on artificial diet. Journal of Agricultural Science 34:168-172.
- Jackai LEN (1995). The legume pod borer *Maruca vitrata* and its principal host plant, *Vigna unguiculata* (L.) Walp. use of selective insecticide sprays as an aid in the identification of useful level of resistance. Crop Protection 14:299-306.
- Lee ST, Srinivasan R, Wu YJ, Talekar NS (2007). Occurrence and characterization of a nucleoplyhedrovirus from *Maruca vitrata* (Lepidoptera: Pyralidae) in Taiwan. Biological Control 52:801-819.
- Liao CT, Liu CS (2000). Occurrence of legume podborer *Maruca* testulalis (Geyer) (Lepidoptera: Pyralidae) on cowpea *Vigna* unguiculata (L.) Walp. an its insecticides application trial. Plant Protection Bulletin 42:213-222.
- Liu H, Skinner M, Brownbridge M, Parker BL (2003). Characterization of Beauveria Bassiana and Metarhizium *anisopliae* isolates for management of tarnished plant bug, *Lygus lineolaris* (Hemiptera: Miridae). Journal of Invertebrate Pathology 82:139-147.
- Muhammad A, Malgwi AM, Adamu RS (2017). *Maruca vitrata* (Fabricius) [Lepidoptera: Pyralidae] larval population dynamics as affected by intra-row spacing, sowing dates and biopesticides on cowpea. Journal of the Science of Food and Agriculture 1:352-364.
- Prior C (1992). Discovery and characterisation of fungal pathogens for locust and grasshopper control. In: Lomer, C.J. and Prior, C., eds. Biological Control of Locusts and Grasshoppers. CAB International, Wal lingford, London, 159-180.
- Scholz BCG, Monsour CJ, Zalucki MP (1998). An evaluation of selective Helicoverpa armigera control options in sweet corn. Australian Journal of Experimental Agriculture 38:601-607.
- Singh B, Ehlers J, Sharma B, Freire Filho F (2002). Recent progress in cowpea breeding. Fatokun CTSA, Singh BB, Kormawa PM. Ed. Challenges and Opportunities for Enhancing Sustainable Cowpea Production, IITA, Ibadan, Nigeria pp. 22-40.
- Sokame BM, Tounou AK, Datinon B, Dannon AE, Agboton C, Srinivasan RB, Pittendrigh R, Tamò M (2015). Combined activity of *Maruca*

vitrata multi-nucleopolyhedrovirus, *Mavi*MNPV, and oil from neem, *Azadirachta indica* Juss and *Jatropha curcas* L., for the control of cowpea pests. Crop Protection72:150-157.

- Srinivasan R, Ghosh SK, Nguyen TTH, Lin MY, Vu Thi TT, Le Duc K, Vu Manh H (2014). Susceptibility of legume pod borer (LPB), *Maruca vitrata* to microbial control agents. The Philippine Agricultural Scientist 97(2):199-203.
- Tamo M, Ekesi S, Maniania NK, Cherry A (2003). Biological control: a non-obvious component of integrated pest management for cowpea.
 In: Biological control in integrated pest management systems in Africa. Neuenschwander P. Borgemeister C. and Langewald, J (Eds.). Wallingford pp. 205-309.
- Tamo M, Srinivasan R, Dannon E, Agboton C, Datinon B, Dabiré C, Baoua I, Ba M, Haruna B, Pittendrigh B (2010). Biological Control: a Major Component for the Long-Term Cowpea Pest Management Strategy. Paper presented at the 5th World Cowpea Research Conference, 27 September to 1 October 2010, Dakar, Senegal 26 p.

- Tanada Y, Kaya HK (1993). Insect pathology. Singh S. R., Van Emden H. F. & Taylor T. A., (Ed.), Academic Press, London, United Kingdom 666 p.
- Toffa Mehinto J, Atachi P, Douro Kpindou OK, Tamo M (2014a). Pathogenicity of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* on larvae of the legume pod borer *Maruca vitrata* (Lepidoptera: Crambidae). Journal of Agricultural and Biological Science 9:55-64.
- Toffa Mehinto J, Atachi P, Douro Kpindou OK, Dannon EA, Tamò M (2014b). Mortality of *Maruca vitrata* (Lepidoptera: Crambidae) larval stages induced by different doses of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*. International Journal of Advanced Research 4:273-285.