Journal of Entomology and Zoology Studies
Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2023; 11(6): 120-129

Received: 05-10-2023
Accepted: 09-11-2023

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Evaluation of the effect of the fungus *Beauveria bassiana* (Balsamo) Vuillemin, on fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctridea) larvae on five maize varieties in the laboratory and screen house

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DOI: https://doi.org/10.22271/j.ento.2023.v11.i6b.9268

Abstract
Beauveria bassiana (Balsamo) Vuillemin, an entomopathogenic ecologically friendly fungus was tried in the laboratory and at the screen house to test for the potency of the fungi against varying stages of the fall armyworm larvae. Five IITA maize varieties were used to determine the efficacy of ten isolates of *B. bassiana* and the experiments were arranged in a Completely Randomized Design for the laboratory experiment and Randomized Complete Block Design for the screen house trial. Two hundred microns of three level dilutions (101, 103 and 105) of the ten isolates of fungi that had been identified using morphological and molecular tools were tried on the second, third and fourth instar larvae of the fall armyworm in a 12cm diameter feeding chamber to determine the potency of the fungus to immobilize and kill the larvae based on daily mortality checks for the laboratory analysis while for the screen house, the second instar larvae treated with the ten isolates of the *Beauveria bassiana* at concentration levels 101, 103 and 105 were introduced into the whorl of individual stand of four week old maize planted in rows of ten plants and observed for damage over a period of seven days and rated using IITA visual rating scales for leaf damage assessment. The experiments were replicated three times and data collected were analyzed using SPSS 2.0 at 5% level of significance. All the isolates were very potent against the larvae of the FAW at higher concentrations and lower dilutions for both experiments.

Keywords: *Beauveria bassiana*, *Zea mays* L., IITA, SPSS, FAW

Introduction

Maize (*Zea mays* L.) is widely grown cereal throughout the world and has the highest production tonnage of all cereals with 990 million tones being produced in 2020 (Index box, 2022) [15]. World maize consumption is estimated to be more than 116 million tonnes with 30% and 21% of consumption occurring globally and in Sub-Saharan Africa (SSA) respectively (IITA, 2021). According to the United States Department of Agriculture (USDA) ranking for the year 2022, Nigeria ranks the second largest producer of maize for Africa with production of 12,500 metric tons after South Africa, the largest maize producer at 17,300 metric tons. The production of maize in Nigeria rose by 16 percent in 2022 in comparison to the year 2021 as a result of the Central Bank of Nigeria policy that stopped supplied of foreign exchange for the importation of maize (USAD, 2022). This requires the enhancement of maize production by ensuring that yields obtained from the cultivated land is harnessed maximally to sustain and manage the ever increasing population of the country, particularly since land, a major important medium for cultivation is a scarcity natural resource (Obayelu, *et al.*, 2019) [20] that is being taken over by infrastructural development cannot be expanded.

Climate change is a major factor that has resulted in variations in all environmental and climatic factors that affect crop production (Adunola *et al.*, 2021) [1] particularly for maize production where sudden break in rainfall pattern affects all developmental stages of the crop. Also, the ever increasing world population, Covid-19 pandemic and in recent times the economic recession is gradually eating up the economies of the nations of the world (Dushime and Osele, 2021) [7], Nigeria inclusive, where even the cost of cultivation of an acre of land has tripled, necessitates the protection of what is being produced right from the farm, that is to minimize damage by pests and diseases to ensure increased food production and consequently eradication of hunger (FAO, 2017) [10].

The fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctridea) is considered a principal pest of maize with a peculiar host specificity, it has a special preference for young maize leaves but occasionally infests the ears and can make the maize more susceptible to aflatoxin presence (FAO, 2018) [12]. The insect causes heavy damage on maize leaves directly and yield losses of 39% to over 70% has been reported (Bhusal and Bhattarai, 2019) [3]. The fall armyworm is one of the most wide spread pests in Africa (Adunola *et al*., 2021) [1] and can have several generations per year, it can fly up to 100 km per night and its population continues to build up as it finds more host plants to multiply on especially in the absence of the complex of natural biological enemies (FAO, 2018) [12]. The larval stages particularly the second, third and fourth instar larval stages are the most deadly stages of the fall armyworm that cause more economic damage being the most active stages when the larvae feed the most (FAO, 2018) [12]. They tartar leaves and shoots reducing the surface area of leaves available for photosynthesis, causing lodging and death of young seedlings (Subba, 2019) [26], they even provide openings for infections by pathogens (Prasanna *et al*., 2018) [22].

The increase in the demand for organically produced foods, the adoption of ecologically oriented pest control methods and the need to protect the environment and human health has necessitated the use of biological control agents. Entomopathogenic fungus is one of biological control agents that serve as a useful alternative for pest management and control instead of the harmful chemicals (Aktar *et al*., 2009) [2] that leave residues that are toxic to life and result in complicated health conditions on crops. *Beavueria* spp *is* an entomopathogenic fungus that acts as a parasite on various arthropods and has been researched worldwide (Fancelli *et al*., 2013) [9] for decades in the management of susceptible pests. It immobilizes and cause death of various stages of the arthropod by growing through the cuticle to cause disease (Sinha *et al*. 2016) [27]. *B. bassiana* is being researched as an entomopathogeic fungus to manage pests infestations, Idress *et al*.,2022 reported that the fungus contains a variety of secondary metabolites that are able to causes a disruption of insects structural integrity (Tefera and Pringle, 2003) [24] and result in the death of susceptible athropods.

Its host-specificity is quite high, usually restricted to a few closely-related insect species. It does not affect other groups of insects (natural enemies), plants, animals or humans (FAO, 2018) [12], so it is generally considered safe as an insecticide. As a matter of fact, the use of *B.bassiana* strain R444 has been registered as biopesticide for the control the fall armyworm in barley, sweet corn, sorghum, tomato and wheat in South African (FAO, 2018) [12].

The need for effective management and control for the fall armyworm using ecologically safe method with the potential to be sustained over time makes this study to be aimed at investigating the effectiveness of ten isolates of *B. bassiana* to immobilization and causing mortality on larval stages of the fall armyworm using five maize varieties.

Materials and Methods

Study site

The experiment was carried out at the insect rearing unit of the Entomology laboratory and screen house 13 of International Institute of Tropical Agriculture (IITA), Ibadan, Oyo state Nigeria.

Ten isolates of entomopatogenic fungus, *B. bassiana*, morphologically identified and characterized molecularly cultured from soil samples collected randomly from various locations in Ondo, Osun and Oyo states of the southwest Nigeria was used for the experiment. The maize varieties (TZB-SR, DTSTR-TSYN, PVA SYN 13, SYN-YFZ and PVA SYN 6) were obtained from the Maize Improvement Unit of IITA, Ibadan.

Laboratory experiment

The five maize varieties were planted in pots in the screen house for four (4) weeks and the leaves were used for the laboratory experiment. Maize leaf of 6 cm long was cut and placed in a well labeled plastic feeding chamber of 12cm diameter. Randomly selected second, third and fourth instar larvae of the fall armyworm were introduced into the chamber (Reinert *et al*., 1997) [23]. Two hundred (200) micron spore at three levels of dilutions (10^1, 10^3 and 10^5), based on the spore counts concentration of conidia in each suspension estimated using Neubauer Heamocytometer of the different isolates of the *B. bassiana* were added directly on the larva and then covered. Distilled water was sprayed in the chambers for constant humidity (Monir, 2019) [18] and they were placed on a bench at room temperature in the laboratory. The experiment was arranged in a completely randomized design with three replications. Larvae were observed daily for larval mortality and the 6cm maize leaf used to feed the larvae was changed daily for each chamber during observations. Fungal infection was confirmed from representative cadavers by culturing on Saubaroud Maltose Agar (SMA) (Difco, Detroit, MI) Petri plates in the laboratory.

Screen house experiment.

The screen house experiment was arraigned in a Randomized Complete Block Design with three replications. At four (4) weeks after planting, the stands were properly labeled and second instar larvae treated with *B. bassiana* at concentration levels 10^1, 10^3 and 10^5 were introduced into the whorl of individual maize stand and observed over a period of seven days. The rate of leaf damage as a result of feeding by the larvae was estimate using the IITA fall army worm visual rating scale for leaf damage assessment (Table 1).

All data collected were subjected to analysis of variance using SPSS 2.0.Significant means were separated using 5% level of significance.

Table 1: Visual rating scales for leaf damage assessment

Scale	Description
0	No visible leaf damage
1	Only pinhole damage on leaves
2	Pinhole and shot hole damage to leaf
3	Small elongated lesions (5–10 mm) on 1–3 leaves
4	Midsized lesions (10–30 mm) on 4–7 leaves
5	Large elongated lesions (>30 mm) or small portions eaten on 3–5 leaves
6	Elongated lesions (>30 mm) and large portions eaten on 3–5 leaves
7	Elongated lesions (>30 cm) and 50% of leaf eaten
8	Elongated lesions (30 cm) and large portions eaten on 70% of leaves
9	Most leaves with long lesions and complete defoliation observed

Source: IITA, Ibadan Nigeria

Results

Laboratory experiments

In the laboratory experiment, all the ten isolates of the *B.*

bassania were potent against the armyworm larval activities for all the maize varieties (Figure 1), leaf damage was highest and most evident on the control, however, the fungus at various dilutions was potent, mildly potent and very potent (Figure 2) for the maize varieties A, B, C and E as indicated by the level of leaf damage, inactivity and death of the larvae. The leaves of the maize variety D was the most susceptible and highly damaged particularly at higher dilution levels where leaf damage was most pronounced (Figure 2).

All the isolates tested had significant effect on the larvae compared to the control causing reduced feeding, immobility, death and even induced metamorphosis in some instances. There were significant interactions among the treatment, maize variety and the dilution indicated by the death of the larvae (Table 2) at 0.05% level of significance. Though varying one to another, there was no significant difference between the levels of dilutions of the *Beauveria* isolates and the maize varieties, between the *Beauveria* isolates and the maize variety and between the levels of dilutions and the maize varieties (Table 2). There was significant difference in the interaction of the *B. bassiana*, maize variety and the dilution on the number of days before the death of the larva, but the numbers of days had no significant effect on the maize variety and the dilution ratios. There was this white mucardine substance on the dead larvae that upon culturing and morphological characterized proved to be the *B. bassiana* fungus (Plates 1 and 2). Another anomaly observed in the course of the experiment the early pupating, so that some of the larvae stopped feeding by the sixth day and by the fourteenth day, the larvae that were not dead had metamorphosed to pupa (Plate 3, and 4), this is contrary to what was observed in the control where the first pupating was observed on the twenty second day.

Plate 1: Dead larvae in the laboratory

Plate 2: Dead larvae with white mucardine substance

Plate 3: Larvae that had stopped feeding from sixth day.

Plate 4: Pupated larvae

Table 2: Mean squares from the Analysis of Variance for larva mortality using the ten B. bassiana at different dilutions on five maize varieties in the laboratory

Source of Variation	Degree of Freedom	Mean Square
Isolate	9	0.140**
Dilution	4	0.091
Variety	4	0.060
Isolate*Dilution	20	0.072
Isolate*Variety	36	0.061
Dilution*Variety	12	0.038
Isolate*Dilution*Variety	80	0.093
Error	311	0.056
Total	476	

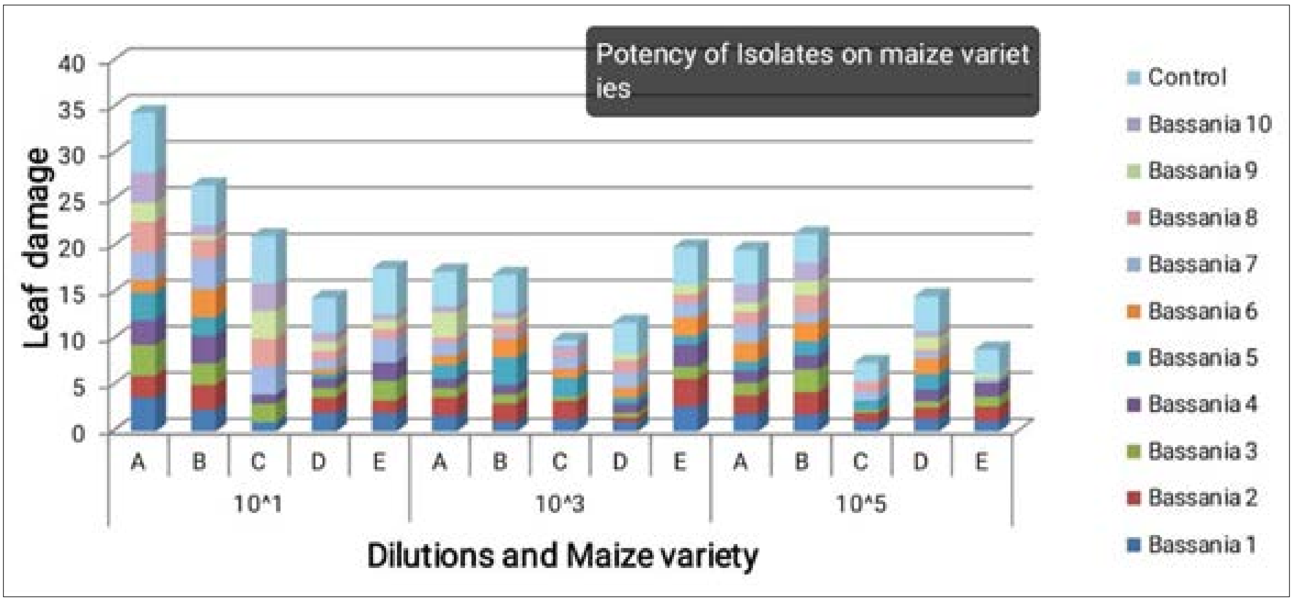

Fig 1: Effect of isolates on maize varieties.

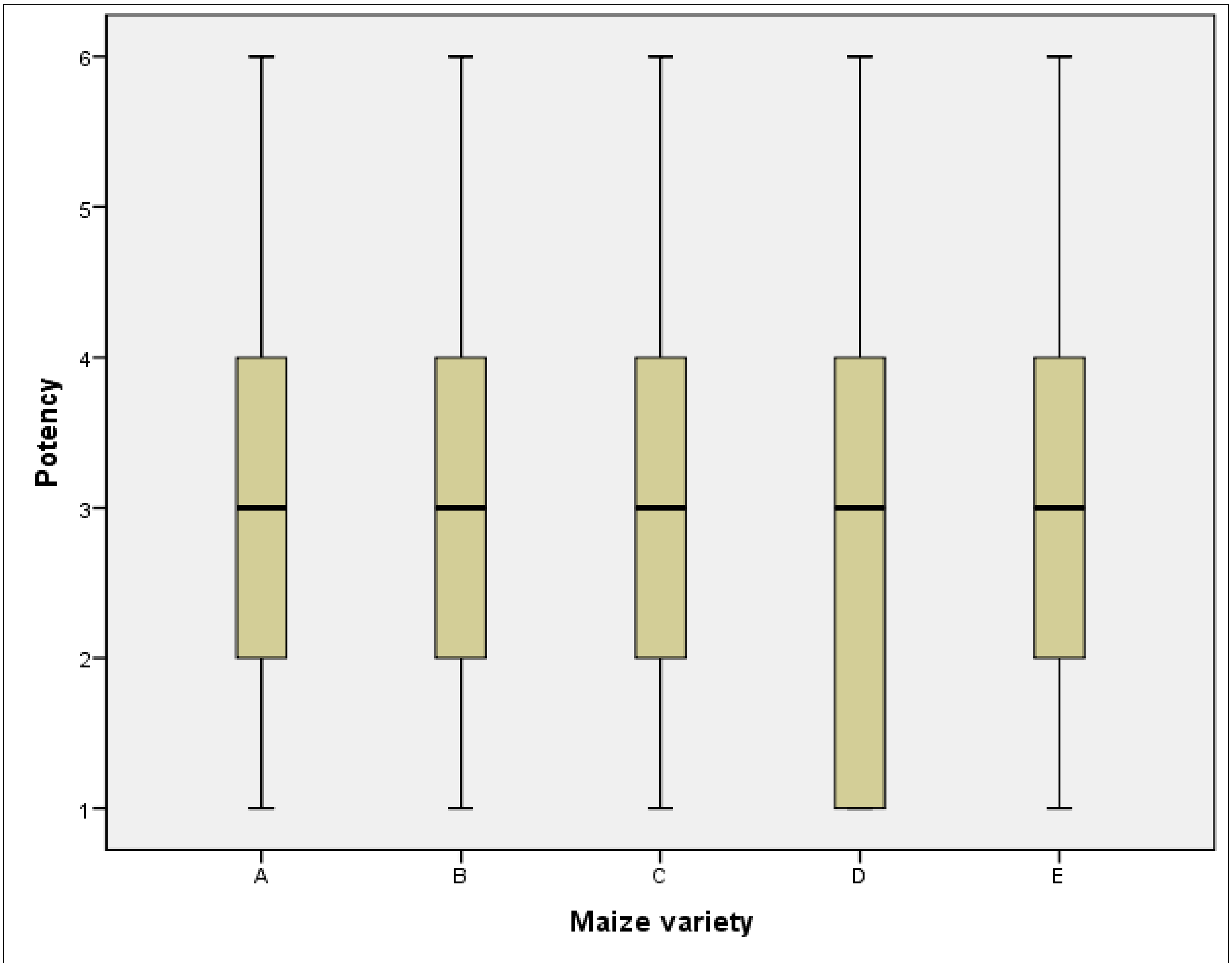

Fig 2: Effect of maize variety on larva mortality.

Dialogue Box

1. Not potent
2. Potent
3. Mildly potent
4. Very potent
5. Strongly potent
6. Highly potent

Screen house trial

The *B. bassiana* isolates were very effective to immobilize and kill the larvae of the FAW in the screen house (Plate 5) and the leaf damage assessment as a result of feeding by the second instar larvae is presented in Figures 3 to 12.There was significant larvae mortality for all the five (5) maize varieties tested with the ten (10) isolates of *B. bassiana* compared with the control, however, there were variations in the effect of the isolates, level of dilutions and the maize varieties which were not significantly different.

Plate 5: Dead larva treated with *B. bassiana* and tattered leaf of control due to larval activities

Figures 2 to 12: Effect of different levels of isolate of *B. bassiana* isolates on leaf damage of maize

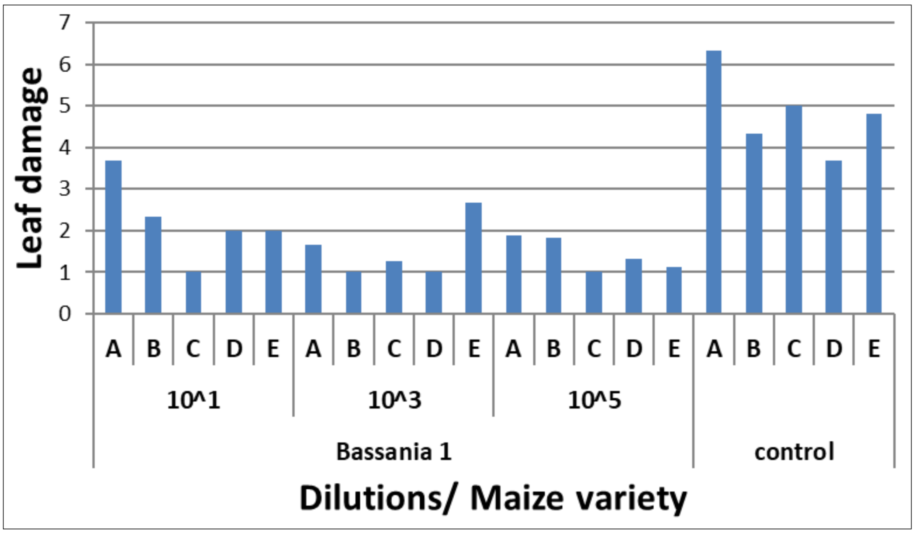

Fig 3: ***B. bassiana*** **isolate 1**

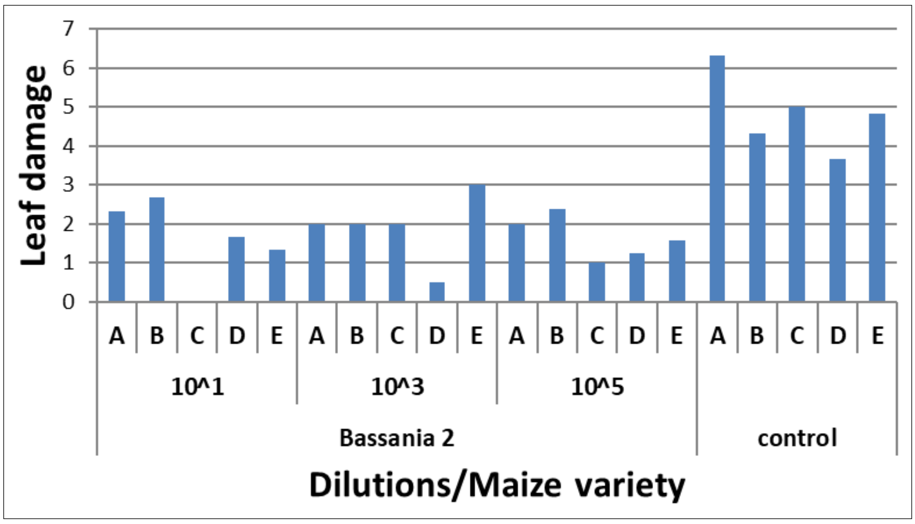

Fig 4: *B. bassiana* isolate 2

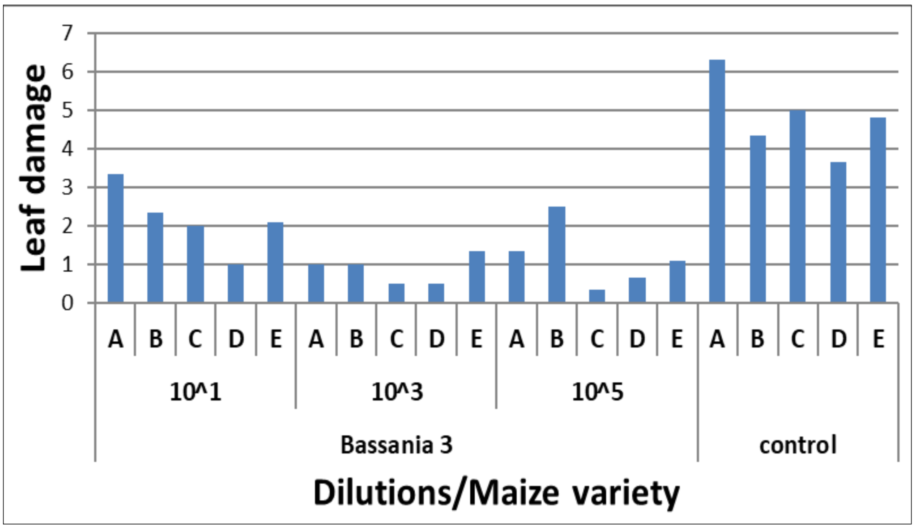

Fig 5: *B. bassiana* isolate 3

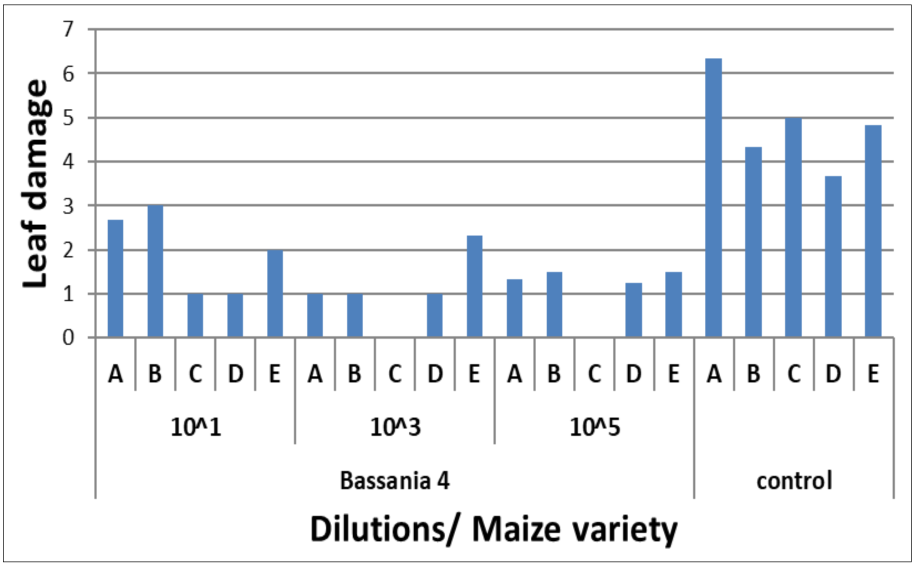

Fig 6: *B. bassiana* isolate 4

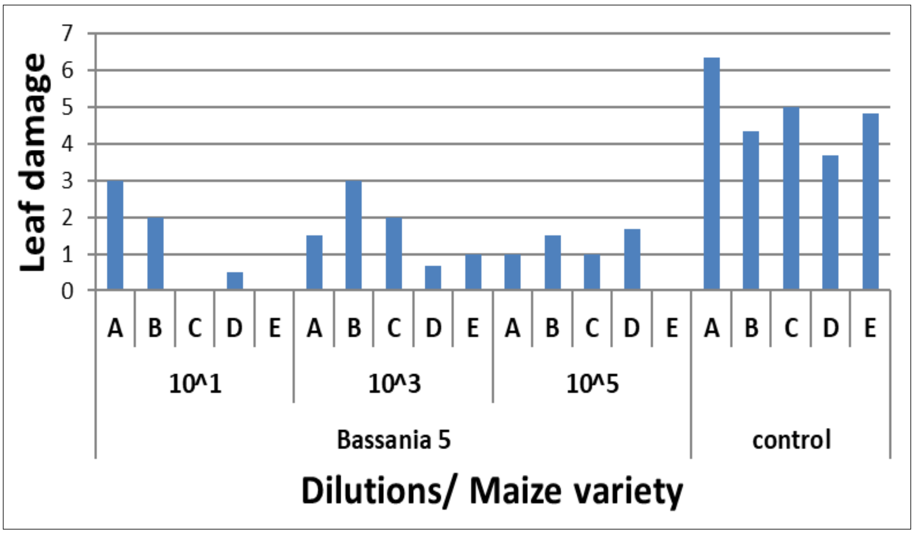

Fig 7: *B. bassiana* isolate 5

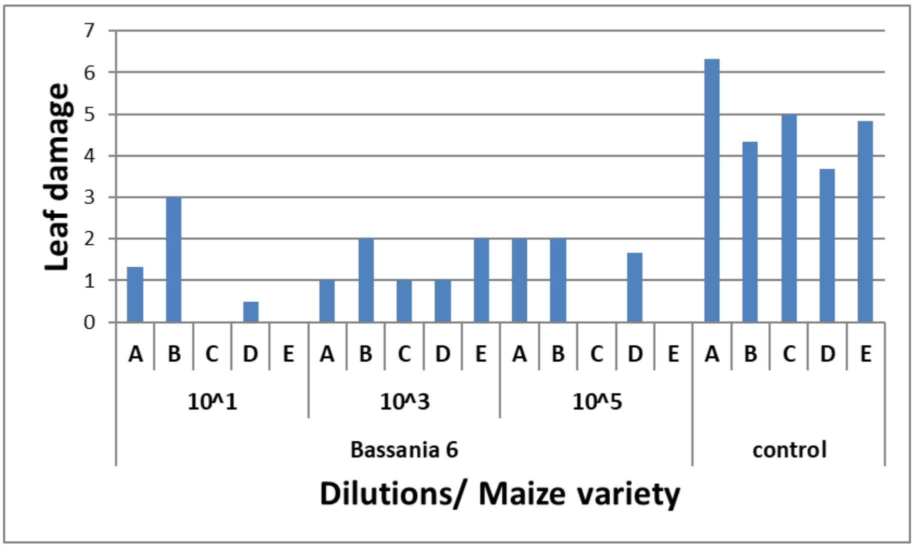

Fig 8: *B. bassiana* isolate 6

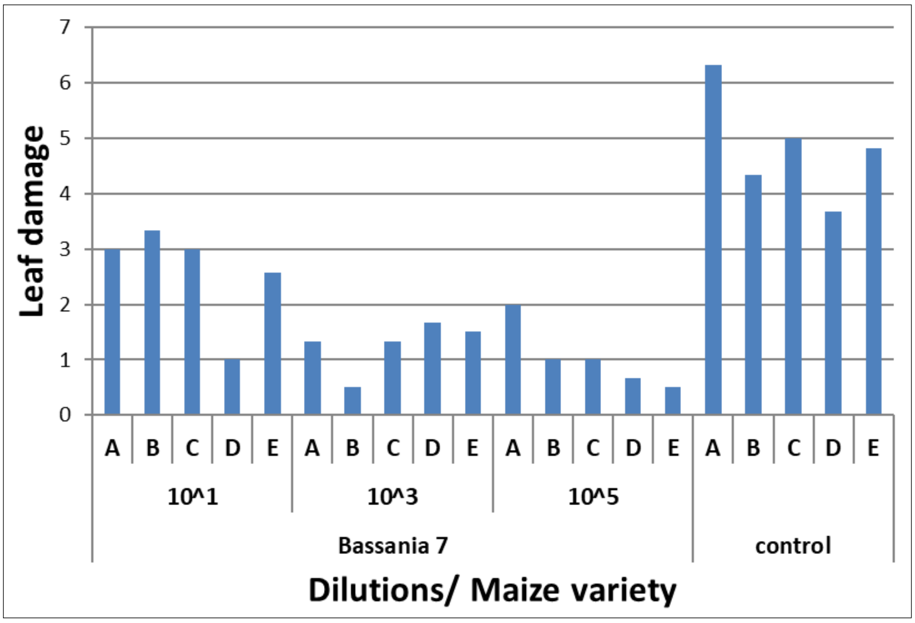

Fig 9: *B. bassiana* isolate 7

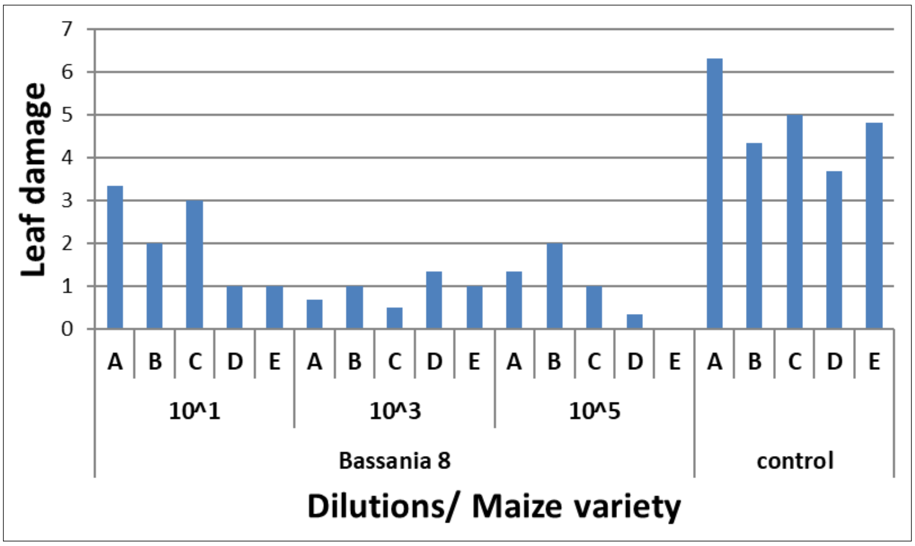

Fig 10: *B. bassiana* isolate 8

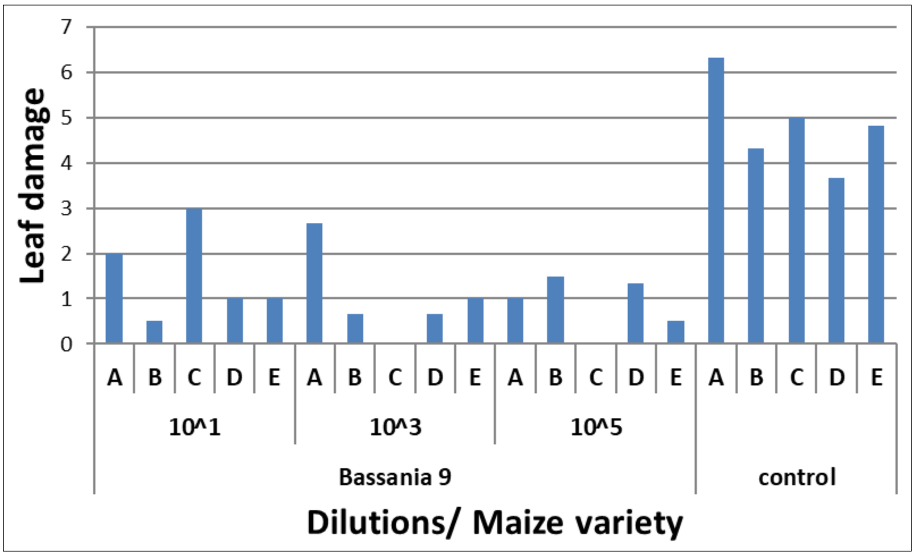

Fig 11: *B. bassiana* isolate 9

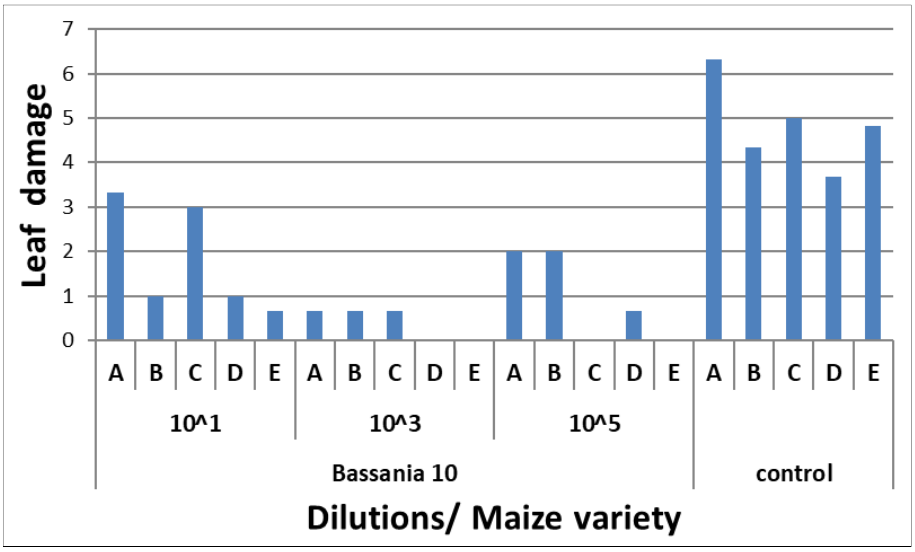

Fig 12: *B. bassiana* isolate 10

The results for the screen house trial showed that the *B. bassiana* was most effective in varieties C and D for isolate 1 and A and B were most damaged by the larvae although with no significant difference in the variations among the varieties, the least level of the three dilutions tried (10^5) was found to be most effective to cause mortality of the armyworm larvae for isolate 1. For isolate 2, variety E was the most damage by the armyworm larvae feeding while isolate C and D were the least damaged with C not having any visible damage, the highest level of dilution (10^1) was observed to be most effective for the management of the fall armyworm. For isolate three, dilution level 10^3 was tested to be most effective while the dilution level 10^1 was least effective and the most damage was observed in the A maize variety. For isolate 4, most damage was observed in maize varieties A and B while the least damage was on varieties C and D for all levels. With isolate 5, there was no visible damage by the fall armyworm on varieties C and E at dilution levels 10^1 and most damage was observed on variety B at dilution level 10^3. Most damage was observed in variety B at dilution level 10^1 while no visible damage was observed on maize varieties C and E at dilution levels 10^1 and 10^5 respectively. Variety B at dilution level 10^1 was most damaged and least damage was observed in same variety B at level 10^3 isolate 7. For isolate 8, there was no visible leaf damage for maize variety E at dilution level 10^5 and most damage was observed on maize variety A at dilution level 10^1. There was no damage on maize variety C at dilution levels 10^3 and 10^5 while most damage was observed on maize C, A and B respectively with results showing that isolate was most effective on variety E. Most damage was observed in maize A at dilution level 10^1 and there was no damage to the leaves of varieties D and E at dilutions 10^3 and C and E at dilution 10^5. Consequently, isolates 2, 4, 6, 9 and 10 were the most effective of the strains having leave damages less than 3 using the IITA fall armyworm visual rating scale for leaf damage assessment.

The higher the concentration or lower the level of dilution the more effective the isolates but this cannot be said for the screen house experiment. There are indications as observed in most of the varieties of maize tested and levels of dilutions used that factors other than the varietal differences in the maize plants tested and levels of dilutions affected the results of the screen house experiment as evident in maize variety B and isolate 7 at dilution levels 10^1 and 10^3.

Discussion

The *B.bassiana* was able to effectively control the FAW in the laboratory and the screen house. This effectiveness was activated by the use of a surfactant that ensured the fungus was able to attach itself to the bodies of the larva to effect penetration and infection (Keswani *et al*., 2013) [17]. Husseini, 2019 established the capacity of *B. bassiana* to induce acceptable mortality rate for armyworm larvae in sugar beet both in the laboratory and on the field reducing the armyworm population, in this study, the larvae treated with *B. bassiana* were observed to be very active when first introduced into the experimental chamber containing the maize leaf and even fed vigorously from the inside of the whorl of the maize for the screen house experiment but soon became sluggish, not feeding and mortality was recorded from the first 24 hours of treatment particularly for the second instar larvae. This can be considered a resultant effect of the penetration of the fungus penetrating into the system of the larvae to infect and instrument a discomfort that distrupts the metabolic activities of the larva (Dubovskiy, 2022) [6]. As the fungus makes its way into the system of the larvae and is able to establish its hypae within the larvae, the larvae has little chance of survive because if means it has been infected (Dubovskiy, 2022, Butt, *et al*., 2016) [6, 4] despite activation of immune responses, this could also account for the high mortality in the second instar larvae where the immune system is not fully established and the forced pupating as an attempt to wage of the effect of the efficacy of the fungus (Butt *et al*., 2016) [4]. The damage on the control was quite visible in the tattering of the leaves. The larvae were weakened with death following from the second to the sixth day, this condition could be attributed to the penetration of the toxic metabolites of beauvericin, bassianin, bassianolide, tenellin, cyclosporin and oxalic acid (Idrees *et al*., 2022 and Dubovskiy, 2002) [14, 6]. These metabolites have insecticidal, antibiotic, cytotoxic, ionophoric, and toxic proteins that are capable of causing mortality (Keswani, *et al*., 2013) [17] and may likely be responsible for the structural disruption described by Tefera and Pringle, 2003 [24] and subsequent germination of the with white mucardine substance the growing from the dead larvae. Some of the larvae that did not die were forced to pupate at less than 20 days into their life cycle, earlier than the normal life cycle of 30-80 days (Capinera, 2017) [5] depending on prevailing condition for the armyworm as a means of resistance to adverse condition of treatment with *B. bassiana.*

There were variations in the response of the larvae to the fungi treatment and these variations could probably be attributed to response of the larvae as a biological agent and its response to its environment.

The non significant difference in effectiveness of the *B. bassiana* on the maize varieties is an indication that treatment with *B. bassiana* for armyworm larvae is potent although more so at higher concentrations or lower dilution, irrespective of the maize variety. It also means that varieties some varieties of maize respond better than other varieties treated with the *B. bassiana* isolates with varying potency using different dilutions of the strain spores, it is required that lower dilution or higher concentrations must be engaged to achieve potency against the fall armyworm.

However, it was observed that as the days go by, the potency of the *Beauveria* was wearing out which means potency is gradually reduced and there is possibility that larvae that are not killed in the first 3-6 days may survive treatment requiring that the treatment be repeated at a frequent interval until the larvae are completely eradicated or when cobs are fully set when the maize would have outgrown the stage that the damage can cause significant losses as the armyworm have preference for young succulent leaves (Capinera, 2017) [5]. Also, aside the preference of the FAW larvae for young succulent leaves, its trans-boundary characteristic (Otim, *et al*., 2021) [21] such that eggs can be laid by adults consistently through the life cyle of the maize crop and the wide host range (Montezano *et al*., 2018) [19] of the insect makes availability of alternate host help sustain the larvae through short periods of unfavourable conditions.

Conclusion

All the ten isolates tested were effective to immobilize and kill the fall armyworm larvae at second, third and fourth instar levels at high concentrations both in the laboratory and the screen house at levels of dilutions and irrespective of the maize varieties, with no significant difference for the ten isolates tried. This implies that as an eco friendly entomopathogenic fungus, the isolates can be made available to farmers in powder and solution form for field application to protect their crops from the fall armyworm damage at specified recommendations.

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