EVALUATION OF GROWTH CHARACTERISTICS OF SELECTED *BEAUVERIA BASSIANA* ISOLATES UNDER LABORATORY CONDITIONS IN KENYA

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Abstract

An evaluation of the growth characteristics of nine B. bassiana isolates was conducted under laboratory conditions. Tests for spore concentration, viability, germination percentage, relative hyphal growth and speed of conidial germination were done. Isolate J59 showed the highest spore concentration of 120 and 114.2 spores per ml during the first and second assay respectively. Isolate J57 had the most viable number of spores in both assays. The highest germination percentage of over 80% was recorded for isolate J57. The relative hyphal growth was highest for the isolateRI under 6 days after inoculation followed closely by isolate J57 in both assays. In all the observed days, isolates BBC and BVT recorded the lowest relative hyphal growth but highest conidial germination at 2 to 10 hours after inoculation. Evaluation of growth is an important prerequisite for evaluation when considering the B. bassiana isolates for control of storage pests.

Key words: *Beauveria bassiana*, spore concentration, viability, relative hyphal growth, speed of conidial germination

1. Introduction

Beauveria bassiana is the most studied to develop biological control agents in agriculture and are available commercially (Khan et al., 2012) being frequently isolated from mycosed insect corpses (Devi et al., 2001), and used as entomopathogenic agent in biological control programs (Wang et al., 2005; Cruz et al., 2006; Dolci et al., 2006).*B. bassiana* colonies are white in colour and have aerial mycelium. Conidia are globose, which are usually larger than 3.5µm in diameter to oval shape (1.5-5.5. X 1-3µm). Conidiophores are single or branched, oblong, cylindrical, or flask shaped bearing laterally or at extremity, vesicles giving rise to porogeneous cells (phialides). Phialides generally are globose, sometimes cylindrical, flash like and curved or straight. It is a naturally occurring entomopathogenic fungus that can easily be isolated from insects, mites, soil and vegetation (Freed et al., 2011a). James et al. (2006) and Hibbet et al. (2007) indicate that taxonomy of fungi has been greatly improved and rigorously documented making it easier to identify important entomopathogenic fungi through their diagnostic characters.

Photographic documentation of microscopic images is now universally accepted as a preferred method of illustration as compared to free hand drawings (Lacey, 2012). A number of Beauveria isolates have been studied due to their potential use as biopesticides (Devi *et al.*, 2001). However, the phenotypic characteristics are neither sufficient to distinguish different Beauveria isolates nor enough to monitor field releases of biocontrol agents (Gaitan et al., 2002; Castrillo et al., 1998). Therefore, this study was carried out to determine the growth characteristics of the selected B. bassiana isolates in laboratory conditions.

2. Materials and methods

2.1. Source of Beauveria bassiana isolates

Seven Beauveria bassiana isolates J29, J35, J36, J39, J57, J59 and RI were sourced as pure plated cultures from biopesticide production companies in Kenya, namely Dudutech Limited and Real IPM Limited. Two B. bassiana isolates were obtained from formulated products, BBC and Beauvitech (BVT) sourced from Genetics Technologies International Limited and Dudutech Limited companies in Kenya respectively.

2.2. Evaluation of quality, safety characteristics of Beauveria bassiana isolates

2.2.1. Spore concentrations

B. bassiana isolates were allowed to grow until sporulation for 14 days on SDAY media petri dishes. The number of conidia per unit weight of each product was determined by suspending 0.1 g samples taken at random in 10 ml containing 0.05% sterile Tween 20 in water clear glass vials with lids. This was vortexed for 30 seconds to produce a homogeneous suspension. The number of conidia or spore concentrations for each isolate was determined using a haematocytometer at 102 dilution to obtain the concentration of spores/ml. Each vial served as replicate with 3 vials for each isolate.

2.2.2 Viability test

The viability of the conidia was determined by adding 200 µl aliquot of each conidial suspension to 20ml of water agar in 9cm petri dishes. A sterile microscope cover slip was placed on each plate and the plates incubated in complete darkness at 25 ± 2°C for 20 hours. Percentage germination was determined by assessing the number of germ tubes formed among 100 randomly selected conidia on the surface area covered by each cover slip under the light microscope (400X). Germination was considered to have occurred when the germ tube was twice the diameter of the conidium. The treatments were arranged in a completely randomized design with 3 replications. To calculate viability of the cells in propagules per ml, the total haematocytometer counts obtained in spore concentration experiment above were multiplied by germination percentage.

2.2.3 Relative hyphal growth

The conidial suspension (0.2ml) of each isolate was inoculated on SDAY plate and incubated at 22°C for 48 hours. Mycelium discs of 6mm diameter were cut off using a sterile cork borer and placed in the centre of freshly prepared SDAY plates. The diameter of the growing colony (radial growth exceeding 6mm diameter of the discs was measured every 2 days for 10 days on a premarked line with a clear ruler. The treatments were arranged in a completely randomized design replicated three times with each plate acting as a replicate.

2.2.4 Rate of sporulation

The conidial suspension (0.2ml) of each isolate was inoculated on water agar and incubated at 22°C for 14 days. Using a sterile cork borer, 5 discs (4mm diameter) was randomly removed from the culture and placed in 10ml sterile distilled water with 0.01 Tween 80 in test tubes. The discs were agitated for 3 hours in a rotary shaker to suspend the conidia. Conidial concentrations in the aliquots of 0.1ml of 10 fold serial dilutions of the aqueous suspensions were determined using a haematocytometer. The mean conidial counts per volume of conidial suspension was calculated for each isolate. The treatments were arranged in a completely randomized design replicated three times with each plate acting as a replicate.

2.2.5 Speed of conidial germination

The conidial suspension (0.2ml) of each isolate was inoculated on SDAY broth and incubated at 22°C for 48 hours. Germination was assessed thereafter bi-hourly ending at 10 hours. The treatments were arranged in a completely randomized design replicated three times with each plate acting as a replicate.

2.4 Statistical Analysis

Analysis of variance (ANOVA) was carried out on the quantitative data after cleaning and tabulation using SAS Version 9.1 statistical software and tested for significance at 99% level of confidence. The treatment means were then separated using the Fishers Protected LSD where significant difference was detected.

3.0. Results

There were significant differences ($P \leq 0.01$) between the isolates on the spore concentration for both assays as shown in Table 3.2 below. Isolates J59, BBC and J35 had the highest number of spores with a mean of 120, 110 and 110 spore counts respectively during the first assay. Isolate J29 had the lowest spore count of 68. The other isolates had a spore count range of between 80 and 100 as shown on the Table 3.2 below. During the second assay, the spore counts differed significantly ($P \leq 0.001$) among the different isolates where J59 showed the highest (114.2) while the lowest was recorded on isolate J29 with a spore count of 65.28 per milliliter (Table 1).

3.1. Viability

The viability of the isolates differed significantly ($P \leq 0.01$) for both assays (Table 3.3). During the first assay, isolate J57 had the highest viability (86.37 cfu/ml) and RI (86.07 cfu/ml) while BBC showed the lowest viability with 79.81 cfu/ml. During the second assay, the same trend as that of the first assay was observed with isolate J57 showing the highest viability and BBC having the lowest viability (78.21 cfu/ml) where isolate J36 and J59 had insignificantly lower viability as that of isolate BBC (Table 2).

3.2. Relative hyphal growth

The hyphal growth observed from the first day to the tenth day showed significant differences ($P \leq 0.01$) on the radial length of the isolates for both assays (Fig. 1 and 2). Isolate RI isolate showed the highest growth rate at all the days except at day ten where the J57 isolate had the highest growth. The latter isolate had shown insignificant hyphal growth compared to RI at day nine. BBC and BVT isolate had the least radial hyphal growth at all the stages with BVT showing lowest from day five. The growth of the hyphae of J29, J35, J36, J39, J57 and J59 was intermediate in all the days as shown in Figure 3.3 during the first assay. In the first 6 days after inoculation, RI had the highest relative hyphal growth then at 7 days after inoculation, isolate J57 which showed significantly the highest hyphal growth thereafter. At the same period of 10 days, the commercial isolate BVT showed the lowest relative hyphal growth just significantly below the other commercial isolate BBC.

During the second assay, RI isolate consistently showed the highest relative hyphal growth from the first day to the tenth day. As observed during the first assay, the hyphal growth was lowest on the already commercialized isolates BVT and BBC.

3.3. Rate of sporulation

There were significant differences at ($P \leq 0.01$) between the mean conidial yields per unit weight of the isolates for both assays (Fig. 3). The highest rate of sporulation was recorded in the commercial isolate BVT with a conidial count of 22.17 and 22.28 per 0.1 ml during the first and second assays respectively. On the other hand, the lowest rate of sporulation was recorded on isolate J36 for both assays with a conidial count per 0.1 ml of 16.7 and 14.7 for the first and second assays, respectively.

3.4. Speed of Conidial Germination

The speed of conidial germination between the isolates was significantly different at all the stage of every two hours up to 10 hours during the first assay (Fig. 4) and second assay (Fig. 5). The conidial germination showed significant differences among the isolates where in the first assay, the formulated isolates BBC and BVT had the highest rate of conidial germination at 2 hours through to 10 hours. Isolate J29 and J59 interchangeably showed the lowest conidial germination through the hours after inoculation. During the second assay, the same trend as that of the first assay was recorded with minimal differences observed between the formulated isolates which had the highest conidial germination.

4.0. Discussion

Morphological characteristics are used as the mode of identification of B. bassiana fungus. The conidia were found to be generally globose and grouped in conidiophores. The cadavers checked for mycosis for Koch's postulates tests had white mycelium confirming that B. bassiana was the causative agent for death. The counts for spore production, viability, speed of conidial germination and relative hyphal growth varied significantly among the different isolates in this study.

Li et al. (2003) reported that B. bassiana isolates that produced higher number of spores were generally most virulent. This could suggest that J59 could be the most virulent while J29 the least virulent. The isolates, J57 and RI had the highest viability while BBC recorded the lowest viability. According to Oliveira et al. (2011) and Jin et al. (2013), viability of conidia is of great importance as during the use of entomopathogenic fungi, as they are the most infectious agents commonly used. Efforts to ensure their viability is maintained need to be employed. The poor viability as observed on J29 may be due to abiotic stresses which is often an obstacle to the use of entomopathogenic fungi as commercial products (Glare et al., 2012).

RI consistently had the highest radial growth rate of the hyphae at all the observation hours. BBC and BVT recorded the lowest radial growth rates through the hours. Wraight and Carruthers, (2001) and Liu et al. (2003) reported that relative hyphal growth is important for causing infection through direct penetration of the host cuticle as the faster the colonization of infected host thereby increased virulence. This makes RI and J57 probable excellent candidates for microbial control use.

The conidial germination of BBC and BVT was fastest soon after inoculation while J29 and J59 recording the lowest conidial germination. Hajek and St. Leger (1994) and Varela and Morales (1996) reported that fungal pathogens which have rapid germination and hyphal growth rates can exhibit faster host infection quicker thus superior virulence that makes them more preferred as biological control agents. They explain further that these factors are necessary for successful infection to take place since not all the time are optimal conditions like relative humidity are available.

5.0. Conclusion

This study has shown thatthe growth characteristics of *B. bassiana* isolates varies and thus their evaluation lays a foundation as a prerequisite to determining their virulence against pests for effective management.

Acknowledgments

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Appendices

Table 1. Mean spore concentration (spores/ml) of Beauveria bassiana isolates

Isolate	Assay 1	Assay 2
BBC	109.0ab	106.0ab
BVT	90.3cd	86.4cd
J29	68.0e	65.3e
J35	110.4ab	105.6ab
J36	90.3cd	86.4cd
J39	86.3d	83.8d
J57	79.3de	76.8d
J59	120.4a	114.2a
RI	101.1bc	95.7bc
P-Value	**<0.001**	**<0.001**
L.S.D	**12.4**	**10.3**
CV%	**5.5**	**4.7**

Treatments with different letters within the same column are significantly different at 1% probability

Table 2. Mean viability (cfu/ml) of Beauveria bassiana isolates

Isolate	Assay 1	Assay 2
BBC	79.8b	78.2b
BVT	83.2ab	80.5ab
J29	81.9ab	81.5ab
J35	83.8ab	82.1ab
J36	80.2b	78.6b
J39	82.1ab	80.4ab
J57	86.4a	84.9a
J59	81.1b	79.2b
RI	86.1a	84.3a
P-Value	**<0.001**	**<0.001**
L.S.D	**4.8**	**4.7**
CV%	**2.5**	**2.4**

Treatments with different letters within the same column are significantly different at 1% probability

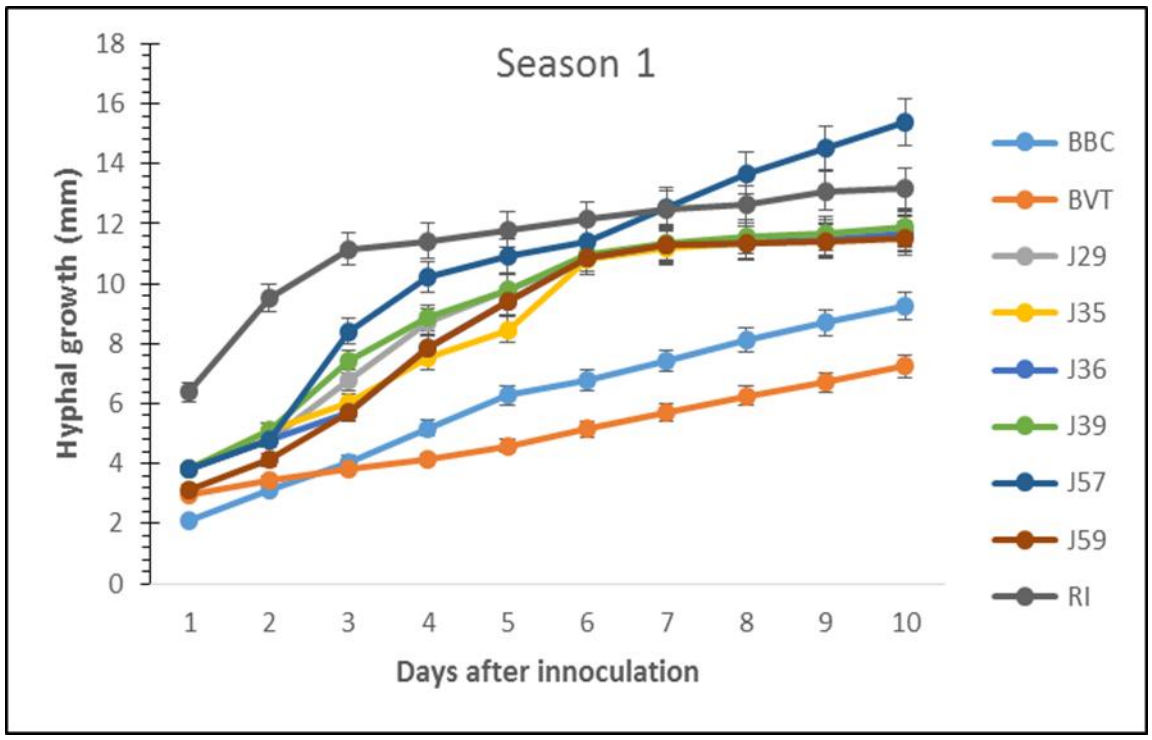

Fig 1: Relative hyphal growth of Beauveria bassiana isolates in assay one

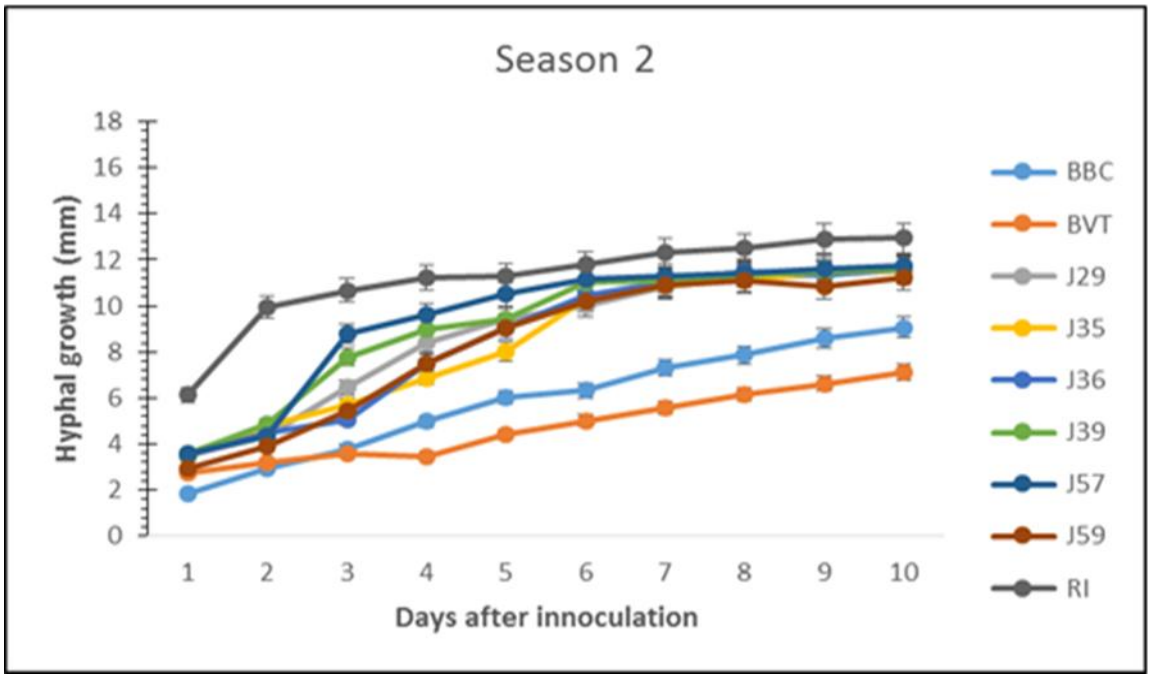

Fig 2: Relative hyphal growth of Beauveria bassiana isolates in assay two

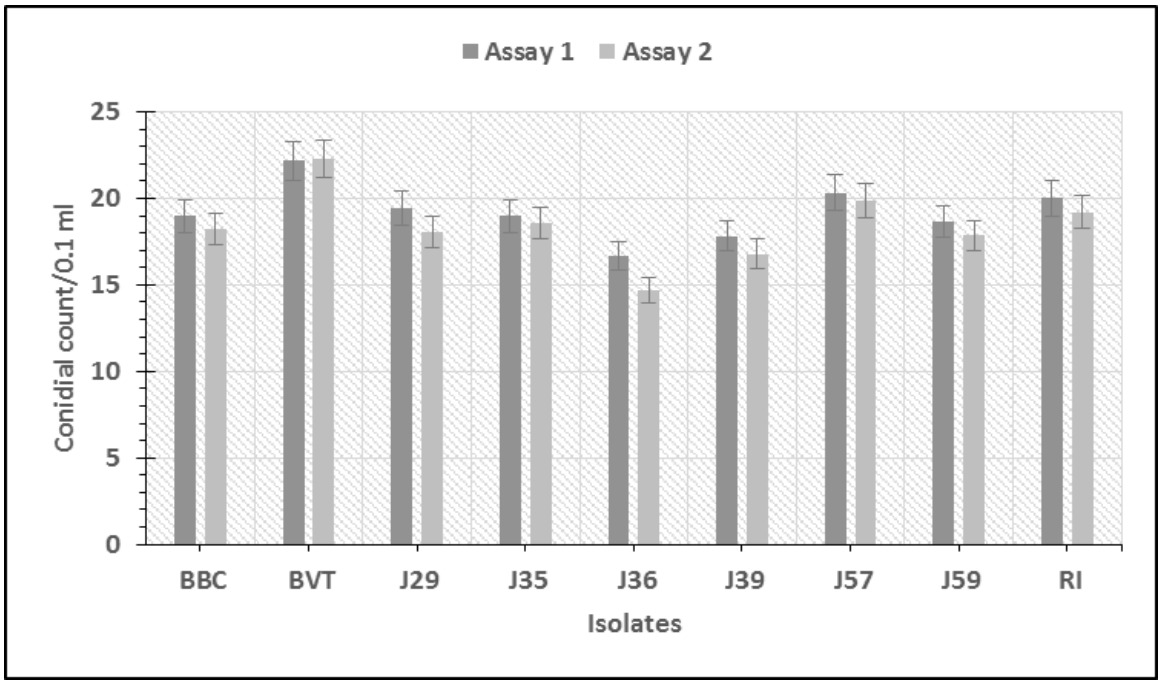

Fig 3: Rate of sporulation of Beauveria bassiana isolates as conidial count per 0.1ml

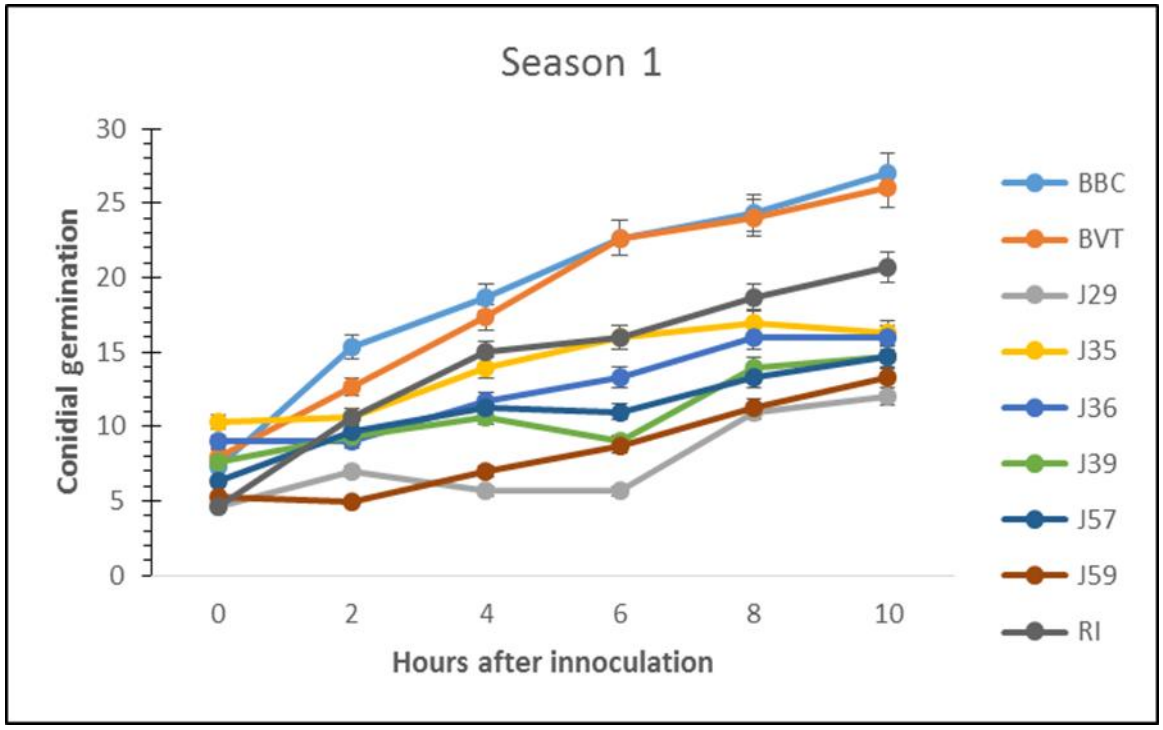

Fig 4: Speed of conidial germination of Beauveria bassiana isolates during assay one

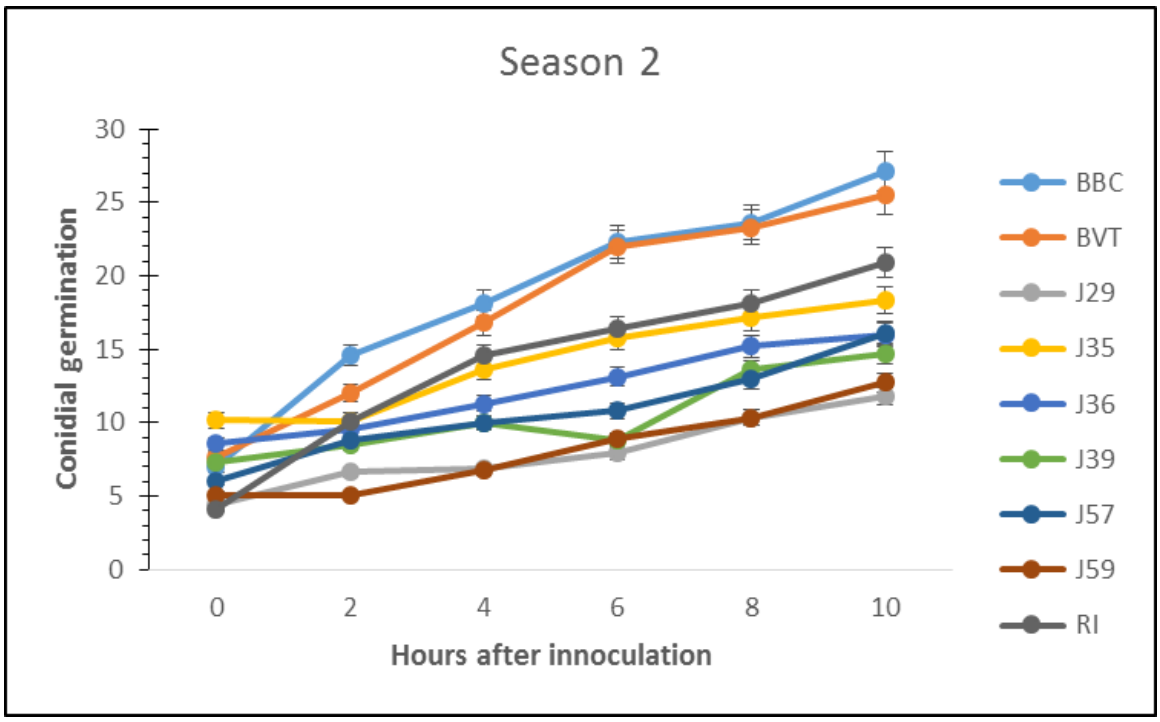

Fig 5: Speed of conidial germination of Beauveria bassiana isolates during the second assay