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RESEARCH ARTICLE

SCREENING THE ABILITY TO CONTROL MESOMORPHUS VILLIGER (BLACK BEAN BEETLE) OF SIX METARHIZIUM ANISOPLIAE STRAINS ISOLATED IN BINH DUONG, VIET NAM

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Abstract

Three types of bio-preparations of six different **Metarhizium anisopliae** strains (Ma-SD, Ma-SAT, Ma-ST, Ma-RS, Ma-RN, and Ma-RM) isolated from soil in Binh Dương province, Viet Nam, including conidia, LIMs and MIXs, were evaluated the acaricidal effects on **Mesomorphus villager** (Black bean beetle). The results of conidia studies showed the high efficacies of Ma-SAT and Ma-ST at 98.64 ± 1.25 percent and 95.23 ± 3.15 percent, in turn. The times to kill half of the studied beetles were observed from the 9th day to the 12th day of the 15-day study process seemed indifferent and late, also without signs of mycosis at the end of the studies. LIMs had low efficacies in the studies, though the LIM of Ma-SAT continued to perform the efficacy at 57.14 ± 3.45 percent among the studied groups after the 15-day study process, moreover, its LIM was the only one killing half of the beetles on day 13th of the study process. The efficacies of MIXs were all higher than 91 percent, among them, Ma-ST, Ma-SAT, and Ma-RS showed their outstanding performance at 96.94 ± 2.49, 98.08 ± 1.21, 98.18 ± 1.38, respectively. Interestingly, all strains were observed to kill half of the studied beetles on day 5th of study time, the soonest one was recorded from the Ma-SAT experiment on day 3rd.

Introduction:-

Mesomorphus viliger (Mv) Blanchard 1853 or dark beetle belongs to Coleoptera: Tenebrionidae: Opatrini. This genus distributes commonly in South Asia, Southeast Asia, Australia, and Africa, in which it is found under stones, in the soils, or the litters of plants such as leaves, rotten tree bark [1], [2], etc. This beetle likely eats the fallen tender leaves of rubber, mango, cashew [3], [4], etc. In Viet Nam, especially Binh Duong province, **Mesomorphus viliger** is named "Black bean beetle" usually appears at the beginning of the rainy season with the high humidity in the air. black bean beetles invade into the house with huge aggregation all daylight, moreover, the invasion becomes stronger when the lights are on in the night. The overpopulation and invasion of Mv have become a serious nuisance for people living beside its habitat, not only does the high concentration of invasive herd also have the ability to secret an irritating and odoriferous quinone to cause mild skin burns. Thus, many solutions such as alcohol-garlic extraction, sprays, and herbs were used by local people to prevent the invasion of these beetles though they showed low efficacies. Moreover, insecticides were proposed to be used for this problem but their safety for humans and animals was considered in the case of the beetles inside the houses. The use of insecticides has a considerable impact on public health due to the contamination of meat and milk with toxic residues and through

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environmental pollution [5], [6]. An alternative for reducing the intensive use of insecticides is to use non-chemical measures such as biological control. For example, the entomopathogenic fungi is a safe and sustainable alternative with minimum risk for vertebrates, humans, and the environment. This fungus infects the insect through contact, invading it through the cuticle and killing it. After the death of the insect, the fungus emerges from the cadaver to produce new conidia [7].

For many years, *Metarhizium anisopliae* (Ma) (Metschn.) Sorokin, a kind of entomopathogenic fungi, has been reported to be used as a biocontrol agent for pest insects and become one of the most widely mycoinsecticides throughout the world. The Ma's infection mechanism begins when conidia, coming from anthropogenically dispersed conidial suspensions or conidia found in the soil, contact to the target insects [8]. Though the entomopathogenic fungus Ma has high toxicity to insects, it basically performs biosafety to vertebrates, humans, and the environment [7]. Unsurprisingly, There were 47 mycoinsecticide products mainly formulated with living propagules of Ma with other additional supporting factors [9], commercially available worldwide [10]. Ma occurs on a wide range of insect hosts with records of 204 naturally infected insect species from seven orders, of which belong to the Coleoptera and especially to soil-dwelling pests insects including over 70 scarab species [11], [12].

The present study aimed to evaluate the acaricidal effects of six different strains of Ma (Ma-SD, Ma-SAT, Ma-ST, Ma-RS, Ma-RN and Ma-RM) isolated from soil in Binh Dương province, Viet Nam against Mv. The results of this study should be documented to produce a potential biosafety agent to control Mv.

Methods:-

***Metarhizium* conidial bio-preparational product producing**:

each of six Ma strains, obtained from soil in Binh Duong province, Viet Nam, was cultured on rice medium including rice 80%, rice bran 10%, and ground corn 10%. 200 g of the cultured medium was distributed into a polyethylene bag, added 40 ml/g of distilled water before autoclaving at 1 atm, 20 minutes. In the initial phase, flasks containing 100 ml liquid medium (20 g glucose, 20 g yeast extract per liter of distilled water) [13] were inoculated with conidia and incubated on a rotary shaker (150 rpm) for 6 days at 30^{o}C. 15 ml of initial biomass was inoculated into each medium that was incubated at 30^{o}C in 5 days before drying at 45^{o}C until the unchanged humidity was observed. The dried fungus biomass was ground and sieved (the diameter of pore size is 150 micrometers) to collect the conidia that were stored at -20^{o}C for the following research [14], [15].

***Metarhizium* liquid bio-preparational product producing:**

1 g of conidia of each Ma strain, obtained from the above process, was grown in an autoclaved flask containing 1000 ml of sterilized Potato - Glucose broth medium at 30^{o}C in 7 days. The biomass was filtered and centrifuged to collect the supernatant that was ready to use as a liquid mycoinsecticide (LIM) [16].

Bioassay procedure:

to determine the efficacy in killing Mv of fungi including conidia, LIM, and conidia mixed with LIM (MIX). In terms of conidia test, 1 ml of conidia suspension of each Ma strain at 10^6 conidia/ml concentration containing 0.05% Tween was used to impregnate filter papers that were placed into a holed-lid plastic box (L x W x H: 13,5 cm x 9 cm x 4 cm), then 100 mature individuals of Mv and 2 g of dry rubber tree leaf (food for beetles) were added into the box. whereas control beetles were placed into the box containing filter paper impregnated only with a 0.5 ml of 0.05% aqueous Tween. The experiment was replicated three times. All treated and untreated groups were observed by daily intervals up to 15 days to detect dead beetles and signs of mycosis [17]. The LIM of each Ma strain was tested the abilities of killing by 5 ml mist spraying of LIM on 100 beetles in the treated boxes, in contrast the control beetles were sprayed by 5 ml of distilled water. The experimental and control groups were replicated 3 times and observed the number of dead beetles day by day interval up to 15 days. Finally, to examine the acaricidal effect of MIX, 5 ml of the suspension of conidia and LIM at 10^6 conidia/ml concentration was sprayed on boxes with 100 beetles, on the other hand, 5 ml of distilled water was used to treat the controls. The experimental and control groups were replicated 3 times and observed the number of dead beetles with signs of mycosis for each day by day interval up to 15 days [18]. The Abbott's formula was applied to determine the efficacy of killing beetles in the experiments [19].

$$H = \frac{X - Y}{X} \text{ x } 100 \text{ (percent control)}$$

Note:

X : the percent living in the control.

Y : the percent living in the treatment.
X - Y : the percent killed by the treatment.

Data Analysis:
the information recorded in the groups under studies and controls was analysed by t-Test for comparing means using STATGRAPHICS CENTURION XV licensed software ($p < 0.05$).

Results And Discussion:-

Effects of different Ma strains' conidia on Mv

The bioassay results showed high effects of entomopathogenic fungi's conidia on the Mv. In the study groups, the percentages of living beetles (Y%) were lower 20 percent after 15 days under studies, in which Ma-SAT and Ma-ST differentially performed abilities to kill Mv to the others with the lowest living percentages including 1.33 ± 1.53 percent and 4.67 ± 3.51 percent, respectively. Whereas the controlled beetles lived healthily till the end of study time with the living percentage of control (X%) was 98 ± 1.0. The low living rates in studies and high living rate in control contributed to the high efficacies in controlling Mv ragging from 80 percent to nearly 99 percent, especially Ma-SAT and Ma-ST with high efficacies were 98.64 ± 1.25 percent and 95.23 ± 3.15 percent, in turn. The times to kill half of studied beetles were observed from the 9th day to the 12th day of the 15-day study process seemed indifferent and late, also without signs of mycosis at the end of the studies.

Table 1:- The average mortality percentages of Mv in different Ma conidia studies and control after 15 days, the average reduction percentages of efficacies calculated with Abbott's formula, and observed time of LC50 values.

No	Strains	Y% (Mean ± sd)	X% (Mean ± sd)	Efficacy (H%) (Mean ± sd)	Time of LC50 (Days) (Mean ± sd)
1	Ma-SD	11 ± 3.6^{b}	98 ± 1.0	88.78 ± 2.51^{b}	10 ± 1.0^{a}
2	Ma-ST	4.67 ± 3.51^{a}		95.23 ± 3.15^{c}	10 ± 1.0^{a}
3	Ma-SAT	1.33 ± 1.53^{a}		98.64 ± 1.25^{c}	9 ± 1.5^{a}
4	Ma-RS	17.33 ± 2.08^{c}		82.32 ± 4.1^{a}	11 ± 1.0^{ab}
5	Ma-RN	6.33 ± 3.05^{ab}		93.54 ± 2.18^{bc}	10 ± 1.0^{a}
6	Ma-RM	19 ± 5.57^{c}		80.61 ± 3.25^{a}	12 ± 1.0^{ab}
Note: Means with the same letters in the same column are not significantly different ($P < 0.05$)					

For an explanation in absence of mycosis, conidia in studies needed more than 15 days to fully grow as natural pathogenic way, in which conidia invaded, germinated, killed the host before causing cuticular degradation. The infection pathway consists on the following steps: (a) attachment of the spore to the cuticle, (b) germination and formation of appressoria, (c) penetration through the cuticle, (d) overcoming of the host response and immune defence reactions of the host, (e) spreading within the host by formation of hyphal bodies or blastospores, i.e.yeast like cells, and (f) outgrowing the dead host and production of new conidia [20]. This pathway in in white grubs or beetles with thick chitin cuticle,as the studied mature beetles, may take 2 - 4 weeks [21]. Moreover, before penetration, germinated conidia of M. anisopliae produce an appressorium, which then forms an infection peg and a penetration plate. Various of hydrolyzed enzymes including proteases, chitinases and lipases, cytochrome P450s, polyketide synthases, and nonribosomal peptide synthetases were released for cuticle-degradation, detoxification and toxin biosynthesis that upgrade their ability to adapt to heterogenous environments [20], [22]. Once inside the host, fungal morphology changes from hyphae to yeast-like blastospores that multiply in the hemocoel and invade other tissues, along with the uptake of nutrients of fungus [23]. Fat body is one of the first tissues colonised by the pathogen and muscle tissue is the last [24]. Besides, Ma secrets acid trehalase for trehalose hydrolysis (trehalose is the main sugar found in insect hemolymph). The fungus accumulates cellular mass and growth continues until the insect is ramified with mycelia. When the internal contents have been consumed, the fungus then develops structures that re-emerge from the insect cadaver, once again producing conidia which disseminate around the mummified insect [25], [26].

Effects of different Ma strains' LIMs on Mv

Results of the studies in table 2. showed weak pathogenic effects of LIMs on Mv management. The observed rates of living beetles were high including studies higher than 60 percent for most treatments, moreover of which Ma-RS and Ma-RN reached to upper than 80 percent, 86.33 ± 4.04 percent and 82.33 ± 5.13 percent, respectively, resulting in recording very low treatment efficacies that were lower 60 percent. Among them, the LIM of Ma-SAT strain continued to perform the potential abilities to control Mv that had been observed with its conidia from the above experiment. The LIM of Ma-SAT strain just left 42 ± 2.65 percent of treated beetles leading to the highest efficacy 57.14 ± 3.45 percent among the studied groups after the 15-day study process, moreover, its LIM was the only one killing half of beetles on day 13 of the study process.

Table 2:- The average mortality percentages of Mv in different Ma's LIM studies and control after 15 days, the average reduction percentages of efficacies calculated with Abbott's formula, and observed time of LC50 values.

No	Strains	Y% (Mean ± sd)	X% (Mean ± sd)	Efficacy (H%) (Mean ± sd)	Time of LC50 (Days) (Mean ± sd)
1	Ma-SD	77 ± 6.08^{cd}	98 ± 1.0	21.43 ± 2.11^{ab}	-
2	Ma-ST	79 ± 3.61^{c}		19.39 ± 1.15^{ab}	-
3	Ma-SAT	42 ± 2.65^{a}		57.14 ± 3.45^{d}	13 ± 1.0
4	Ma-RS	86.33 ± 4.04^{d}		11.91 ± 1.27^{a}	-
5	Ma-RN	82.33 ± 5.13^{cd}		15.99 ± 3.48^{a}	-
6	Ma-RM	65 ± 3^{b}		33.67 ± 2.19^{c}	-
Note: Means with the same letters in the same column are not significantly different ($P < 0.05$)					

Ma showed the synthesis of destruxins (six types), cytochalasins (C and D), and swainsonine as toxic metabolites produced in culture or in vivo [27], [28]. The destruxins of the *Metarhizium* genus and other entomopathogenic fungi are categorised into six major groups: A through F [29]. Some of these compounds are linked to virulence and host specificity in this genus [30]. Destruxins are considered as the main factor of pathogenesis [31] inducing flaccid paralysis, causing cellular alterations and malfunction of the middle intestine, malpighian tubules, and muscle tissues [32], blocking H+ ATPase activity [33] and interacting with Ca2+ channels [31]. The secretion of destruxin A and others could cause an adverse effect on the insect immune system as immune modulators that suppress the insect-host immune response [29], [34]. The secretion of destruxin A could cause an adversely effect in the insect immune response, but not enough to kill the host [35]. Less effective of Ma's secreted secondary metabolites on hosts should be a relevant explanation for weakness in killing Mv of LIMs in this study.

Effects of different Ma strains' MIXs on Mv

The mixture of conidia and LIM (MIX) performed high potency in killing Mv in this study. After 15 days of the study process, the means of living rates in all studies were very low ragging from 1 percent to 7 percent, whilst the controlled Mv lived up to 98 percent. These supported the high efficacies were all higher than 91 percent, among them, Ma-ST, Ma-SAT, and Ma-RS showed their outstanding performance of which efficacies were 96.94 ± 2.49, 98.08 ± 1.21, 98.18 ± 1.38, respectively. Interestingly, all strains were observed to kill half of the studied beetles on day 5th of study time, the soonest one was recorded from the Ma-SAT experiment on day 3rd. In separated experiments, though, conidia showed high efficacies nearly the same as that of MIX, their times of LC_{50} were late. Moreover, this index was not recorded in most of the studied LIMs, exceptionally Ma-SAT strain.

Table 3:- The average mortality percentages of Mv in different Ma's MIX studies and control after 15 days, the average reduction percentages of efficacies calculated with Abbott's formula, and observed time of LC50 values.

No	Strains	Y% (Mean ± sd)	X% (Mean ± sd)	Efficacy (H%) (Mean ± sd)	Time of LC50 (Days) (Mean ± sd)
1	Ma-SD	7.0 ± 1.73^{b}	98 ± 1.0	91.06 ± 1.15^{a}	5 ± 1.0^{b}
2	Ma-ST	3.0 ± 2.0^{a}		96.94 ± 2.49^{ab}	5 ± 1.0^{b}
3	Ma-SAT	1.0 ± 0.0^{a}		98.08 ± 1.21^{b}	3 ± 1.0^{a}
4	Ma-RS	1.0 ± 1.0^{a}		98.18 ± 1.38^{b}	5 ± 1.0^{b}
5	Ma-RN	2.0 ± 1.0^{a}		93.96 ± 4.1^{a}	5 ± 1.5^{b}

6	Ma-RM	1.67 ± 0.58^{a}		95.30 ± 1.47^{ab}	4 ± 0.0^{ab}
Note: Means with the same letters in the same column are not significantly different ($P < 0.05$)					

Though secondary metabolites produced in LIM, including destruxins, cytochalasins, and swainsonine [27], [28] might not be the main factors causing the deaths of Mv, they had abilities to disturb the normal metabolism and reduce the functions of the immune system [35], resulting in weak beetles. The fundamental effects of these bioactive compounds helped conidia to start the infection pathway on the hosts easier and faster than usual leading to the host's sooner deaths. Obviously, the orderly and accumulative effects of bio-compounds in LIM and pathogenic conidia were considered the relevant explanations for outstanding killing activities on Mv.

Conclusion:-

The results of this study demonstrated effects in control Mv (Black bean beetles) of six Ma strains including Ma-SD, Ma-SAT, Ma-ST, Ma-RS, Ma-RN, and Ma-RM. All types of bio-preparations produced from these strains had potential effects on killing Mv to reduce their nuisance on human life. Moreover, the Ma-SAT strain with its conidia, LIM and MIX performed the highest efficacies on reducing the number of Mv compared to the others in the experiments. As a result, though Ma-SAT strain and its bio-preparations should be considered as biological control agents for Black bean beetle, on which more and more investigations have to be carried out to clearly evaluate and ensure their real effectiveness.

References:-

1. S. Sitaramaiah, G. Rama Prasad, and U. Sreedhar, Management of tobacco ground beetle, Mesomorphus villiger with insecticide baits on flue cured Virginia tobacco. Indian Journal of Agricultural Sciences. **69**(9), pp. 660-663, 1999.
2. W. Schawaller, The genus Mesomorphus Seidlitz in Nepal. Faunistische Abhandlungen Staatliches Museum Fur Tierkunde, Dresden. **22**(4), pp. 39-48, 2000
3. Chuliath,V.P. Joseph and S.K. Thomas, Darkling beetles (Coleoptera: Tenebrionidae) of forest sites and agricultural fields in the south Western Ghats (South India). Journal of Insect Biodiversity. **5**(3), pp. 1-12, 2017.
4. V.D. Hegde, B. Lal and K. Chandra, New Records of Darkling Beetles (Tenebrionidae: Coleoptera) from Chhattisgarh, India. Biological Forum – An International Journal. **7**(1), pp. 707-711, 2015.
5. National Institude of Malariology Parasitology Entomology in Ho Chi Minh city (IMPEHCM), Results of investigation and monitoring of black bean beetle in Dong Nai province, Available at www.impehcm.org.vn, accessed November 2021.
6. National Institude of Malariology Parasitology Entomology in Ho Chi Minh city (IMPEHCM), Black bean beetle appears and affects people's lives in Minh Long commune, Chon Thanh district, Binh Phuoc province, Available at www.impehcm.org.vn, accessed November 2021.
7. G. Zimmerman, Review on safety of entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Sci. Tech. **17**(9), pp. 879-920, 2007.
8. H. Hesketh, H.E. Roy, J. Eilenberg, J.K. Pell and R.S.Hails, Challenges in modelling complexity of fungal entomopathogens in semi-natural populations of insects. The Ecology of Fungal Entomopathogens. pp. 55–73, 2010.
9. S.G. de León and T. Mier, Visión general de la producción y aplicación de bioplaguicidas en méxico. Sociedades Rurales, Producción Y Medio Ambiente. **10**(20), pp. 37–63, 2010.
10. M.R.D Faria and S.P. Wraight, Mycoinsecticides and Mycoacaricides: A comprehensivelist with worldwide coverage and international classification of formulation types. Biological Control. **43**(3), pp. 237–256, 2007.
11. K.H. Veen, Recherches sur la maladie due a` Metarrhizium anisopliae chez le criquet pe`lerin. Mededelingen Landbouwhogeschool. Wageningen. **68**(5), pp. 1-77, 1968.
12. D. Leatherdale, The arthropod hosts of entomogenous fungi in Britain. Entomophaga. **15**(4), p.19-435, 1970.
13. N.E. Jenkins and C Prior, Growth and formation of true conidia by Metarhizium flavoviride in a simple liquid medium. Mycological Research. **97**, pp. 1489–1494, 1993.
14. F. Marcos, A.E. Hajek and P.W. Stephen, Imbibitional damage in conidia of the entomopathogenic fungi Beauveria bassiana, Metarhizium acridum, and Metarhizium anisopliae. Biological Control. **51**, pp. 346–354, 2009.
15. C. Ángel-Sahagún , R. Lezama-Gutiérrez, J. Molina-Ochoa, A. Pescador-Rubio, S.R. Skoda and Cruz- C. Vázquez, Virulence of mexican isolates of entomopathogenic fungi (*Hypocreales*: *Clavicipitaceae*) upon

Rhipicephalus Boophilus microplus (*Acari: Ixodidae*) larvae and the efficacy of conidia formulations under field conditions. Vet. Parasitol. **170**(3-4), pp. 278-86, 2010.
16. D.W. Watson, C.J. Geden, S.J. Long and D.A. Rutz, Efficacy of Beauveria bassiana for controlling the house fly and stable fly (*Diptera: Muscidae*). Biol. Control. **5**(3), pp. 405-411, 1995.
17. M. Tavassoli , A. Ownag , S.H. Pourseyed and K. Mardani, Laboratory evaluation of three strains of the entomopathogenic fungus *Metarhiziumanisopliae* for controlling *Dermanyssusgallinae*. Avian Pathology. **37**(3), pp. 259-263, 2008.
18. C. Cruz-Vázquez, J. Carvajal-Márquez, R. Lezama-Gutiérrez, I. Vitela-Mendoza and M. Ramos-Parra, Efficacy of the entomopathogenic fungi *Metarhizium anisopliae* in the control of infestation by stable flies *Stomoxys calcitrans* (L.), under natural infestation conditions. Vet. Parasitol. **212**(3-4), pp. 350-355, 2015.
19. W.S. Abbott, A method of computing the effectiveness of an insecticide 1925. Journal of the American mosquito control association. **3**(2), pp.302-303, 1987.
20. D.G. Boucias and J.C. Pendland, Principles of insect pathology. MA: Kluwer Academic Publishers. Boston. 568pp, 1998.
21. Vilcinskas and P. Gotz, Parasitic fungi and their interactions with the insect immune system. Advances in Parasitology. **43**, pp.267-313, 1999.
22. Q. Gao, K. Jin, S.H. Ying, Y. Zhang, G. Xiao, Y. Shang and C. Wang, Genome sequencing and comparative transcriptomics of the model entomopathogenic fungi Metarhizium anisoplia and M. acridum. PLoS. Genetics. **7**(1), 2011. DOI:10.1371/journal.pgen.1001264
23. Schrank and M.H. Vainstein, Metarhizium anisopliae enzymes and toxins. Toxicon. **56**(7), pp.1267–1274, 2010.
24. S. Schneider, F. Widmer, K. Jacot, R. Kölliker and J. Enkerli, Spatial distribution of Metarhizium clade 1 in agricultural landscapes with arable land and different semi-natural habitats. Applied Soil Ecology. **52**(1), pp. 20–28, 2012.
25. J.J. Boomsman, A.B. Jensen, N.V. Meyling and J. Eilenberg, Evolutionary interaction networks of insect pathogenic fungi. Annual Review of Entomology. **59**(1), pp. 467–485, 2014. DOI:10.1146/ annurev-ento-011613-162054
26. T.M. Butt, C.J. Coates, I.M. Dubovskiy and N.A. Ratcliffe, Entomopathogenic fungi:New insights into host-pathogen interactions. Advances in Genetics. **94**, pp. 307–364, 2016. DOI:10.1016/bs. adgen.2016.01.006
27. H. Strasser, A. Forer and F. Schinner, Development of media for the selective isolation and maintenance of virulence of Beauveria brongniartii. In: T.A. Jackson, T.R. Glare (Ed.). Proceedings of the 3rd International Workshop on Microbial Control of Soil Dwelling Pests. pp.125-13, 1996.
28. Vey, R. Hoagland and T.M. Butt, Toxic metabolites of fungal biocontrol agents. In: T.M. Butt, C.W. Jackson and N. Magan (Ed.). Fungi as biocontrol agents: progress, problems and potential. Wallingsford, UK: CABI International. pp.311-346, 2001.
29. Wang, Q. Kang, Y. Lu, L. Bai and C. Wang, Unveiling the biosynthetic puzzle of destruxins in Metarhizium species. in: Proceedings of the National Academy of Sciences. **109**(4), pp.1287-1292, 2012.
30. Amiri-Besheli, B. Khambay, S. Cameron, M.L. Deadman and T.M .Butt, Inter- and intra-specific variation in destruxin production by insect pathogenic Metarhizium spp., and its significance to pathogenesis. Mycological Research. **104**(4), pp.447–452, 2000. DOI:10.1017/ S095375629900146X
31. R.I. Samuels, A.K. Charnley and S.E. Reynolds, The role of destruxins in the pathogenicity of 3 strains of Metarhizium anisopliae for the tobacco hornworm Manduca sexta. Mycopathologia. **104**(1), pp.51–58, 1988.
32. C. Dumas, P. Robert, M. Pais, A. Vey and J.M, Quiot. Insecticidal and cytotoxic effects of natural and hemisynthetic destruxins. Comparative Biochemistry Physiology. **108**(2), pp.195-2003, 1994.
33. M. Muroi, N. Shiragami and A. Takatsuki, Dextruxin B, a specific and readily reversible inhibitor of vacuolar-type H+-translocating ATPase. Biochemical and Biophysical Research Communications. **205**, pp.1358–1365, 1994.
34. S. Pal, R.J. St. Leger and L.P .Wu, Fungal peptide destruxin a plays a specific role in suppressing the innate immune response in Drosophila melanogaster. Journal of Biological Chemistry. **282**(12), pp.8969–8977, 2007. A. Ríos-Moreno, I. Garrido-Jurado, G. Resquín-Romero, N. Arroyo-Manzanares, L. Arce and E. Quesada-Moraga, Destruxin A production by Metarhizium brunneum strains during transient endophytic colonisation of Solanum tuberosum. Biocontrol Science and Technology. **26**(11), pp.1574–1585, 2016.