Studies on Selection of Entomopathogenic Fungi with High Virulence and Field Persistence for Controlling Common Cutworm, Spodoptera litura

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Title　：Studies on Selection of Entomopathogenic Fungi with High Virulence and Field Persistence for Controlling Common Cutworm, *Spodoptera litura*
（ハスモンヨトウ防除を目指した高い病原力及び定着能力を有する昆虫寄生菌の選抜に関する研究）

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Thesis Summary

The current research was laboratory and field-based experiments with three genera of entomopathogenic fungi *Beauveria, Metarhizium* and *Cordyceps* of which different geographic locations and source of isolations. Entomopathogenic fungal strains belonging to those genera are among the known components of biological weapons in integrated pest management programs. They are naturally safe and eco-friendly biological agents. Their potential of host specificity and ease of handling make them options to substitute synthetic pesticides in pest control. The general outlines of the research was organized in three sections, (1) laboratory based bioassay with 16 entomopathogenic fungal strains against the last instar larvae and pupae of *Spodoptera litura*, (2) screening of 32 fungal strains for *in vitro* heat tolerance at 45^{o}C in 2 h and 4 h exposure period, (3) field persistence assay of selected fungal strains (5 strains) with variable performance of thermotolerance under laboratory for field persistence from pot soils and cucumber leaves in hot and cold season.

The efficiency of fungal stains for controlling agricultural, household, vector insects and dairy farm pests were proved through various post research studies, However, they are sensitive and unstable under field condition. Evaluations on its fitness to withstand a given environmental conditions such as temperature, UV radiations and relative humidity, are important to select strains with better biocontrol efficacy in fields, because these factors have detrimental effect and restricts the use of entomopathogenic fungi in field. Hence screening of the fungal strains done to check the synchrony in potential of fungal strains for *in vitro* heat tolerance and field persistence. All assayed fungal strains come up with certain degree of heat tolerance, where significant variation observed among fungal genera and strains of same species.

Results of *in vitro* heat tolerance assay clarified that colony forming unit counts of fungal strains were seen with the potential tolerance ranging from 0–100% and fungal conidia get distorted with the lengthy periods of heat exposure at the selected temperature level (45^{o}C). The exposure for 4 h at 45^{o}C referred as lethal death point for *B. bassiana* OMNS150429-2, *B. brongniartii* TNO6, *C. fumosorosea* BPS2, *C. javanica* Czy-LP and *M. pingshaense* MS3. A higher dominance in thermotolerance was observed with *Metarhizium* strains in extended heat exposure, while *C. javanica* Czy-LP, *C. fumosorosea* BPS2, *B. brongniartii* TNO6 and *M. pingshaense* MS3 remained

highly susceptible.

Obviously, there are several factors affecting persistence potential of entomopathogenic fungal strains under field conditions like the inherent capacity of the fungal strain, soil type, culture media and others. However, this study was handled regardless of the biotic factors. It focused only temperature to define the relationship between *in vitro* heat tolerance and field persistence. Field observation demonstrated that the three *Metarhizium* species, *M. pingshaense* F2685, *M. pingshaense* MS2 and *M. brunneum* F709 showed significant difference among the assayed strains with survival of CFUs at 7 days and 28 days post inoculations in both hot and cold season trials. Whereas strains of *B. brongniartii* TNO6 and *C. javanica* Czy-LP were vulnerable under field condition. It detailed that direct relationship between the two parameters and concluded *in vitro* heat assay could be used in selection of field persistent fungal strains for hot season applications.

Laboratory based virulence assessment of 16 fungal strains against lepidopteran pest was carried out with the reference to insect pest, *S. litura*. It is one of the devastating pests worldwide. Last instar larvae and pupae were used for bioassays at the concentration of 1 x 10^8 conidia/mL. Data on mortality, percent of mycotized larvae and pupae, and adult emergence were recorded. Fungal strains exhibited varying levels of virulence regarding the treated life stages of the target pest with up to 100% mortality at 10 days post inoculations. Strains of *M. rileyi* Nr4, *M. pingshaense* MS1, *M. brunneum* ARSEF 3294 and *M. pingshaense* ARSEF 8736 with the uppermost average mortality for last instar larvae, however, their virulence against the pupae was comparatively lower. The higher pupal susceptibility was observed with the strains of *B. brongniartii* TNO6, *C. javanica* Czy-LP, *M. brunneum* F709, and *M*. *pingshaense* MS2. Those strains scored virulence ≥90% to pupae and ≥85% to last instar larvae and seem composite strains and could be intensively investigated and developed as potential mycopesticides in integrated pest management programs against *S. litura*. In addition, the reference/commercial *B. bassiana* GHA accounted among the least efficient strains during the lab bioassays.