Chapter 7
Efficacy of a Sudanese Strain of Entomopathogenic Fungus, *Metarhizium anisopliae* Met. Sorokin on Puparia of *Bactrocera dorsalis* Hendel, Under Laboratory Conditions

Abdelgadir M. Abdellah, Ahmed E. M. Hassan, Ahmad A. Eisa, Yousif S. Adam, and Awadallah B. Dafallah

Abstract Fruit flies (Diptera: Tephritidae) are among the main constraints that limit horticultural production in Sudan. The country has an enormous potential for horticultural production, over its wide range of climatic conditions and diverse ecosystems. However, it is threatened by an invasion of exotic fruit flies over its long borders with neighbouring countries, and by its weak interception and quarantine procedures. Although entomopathogenic fungi are known to attack fruit flies, very few efforts have been exerted to confirm their efficacy in integrated pest management (IPM) strategies against fruit flies. The fungus *Metarhizium anisopliae* Sorokin has been found on the dead body of a larva of white grub, *Schizonycha* sp. (Coleoptera: Melolonthidae), at Kenana Sugarcane Farm. A laboratory experiment was undertaken by applying four different concentrations (20×10^6, 10×10^6, 5×10^6 and 2.5×10^6) of the fungus on the pupae of *Bactrocera dorsalis* in order to inhibit adult emergence. The results showed that adult emergence from the pupae of *B. dorsalis* was inhibited by the tested concentrations of *M. anisopliae*. The inhibition increased with increasing concentrations. LC_{50} and LC_{90} values for the fungus were 10×10^6 and 18×10^6 conidia/ml, respectively. The mentioned fungus can be incorporated in fruit fly management strategy.

A. G. M. Abdellah (✉)
Integrated Agricultural Pest Management Research Centre, Insect Taxonomy Unit, Agricultural Research Corporation, Wad Madani, Sudan

A. E. M. Hassan · A. A. Eisa · A. B. Dafallah
Faculty of Agriculture, Department of Crop Protection, University of Gezira, Wad Madani, Sudan

Y. S. Adam
Agricultural Research Corporation, Gezira Research Station, Wad Madani, Sudan

S. Niassy et al. (eds.), *Sustainable Management of Invasive Pests in Africa*, Sustainability in Plant and Crop Protection 14,
https://doi.org/10.1007/978-3-030-41083-4_7

Keywords Fruit flies · Diptera · Tephritidae · Entomopathogenic fungi

7.1 Introduction

Mango (*Mangifera indica* L.) is believed to have been cultivated for about 6,000 years, with its likely native home in Eastern Asia. This species is now grown worldwide, in both hemispheres. The fruit is a good source of fibres and vitamins, and is described as the most favourite and valuable fruit in the world market. Moreover, the fruit is rich in antioxidants and therefore reduces the risks of cardiac disease, and has anti-cancer and anti-viral properties. In Sudan, mango is an important horticultural crop for local consumption and export (Ministry of Industry and Trade 2019). It has economic importance, being produced almost all the year round in different parts of the country (Ministry of Industry and Trade 2019). It also has regional and international demand in markets, being a beloved fruit with great nutritive value and palatable taste. The area cultivated for mango in Sudan is estimated to be about 2,814,000 ha, and its production is estimated at 635,000 tons, annually (Fatima and Dawoud 2017).

Mango is commercially grown in every state – South Kordofan, Sinnar, Blue Nile, West Darfur, South Darfur and Kassala (Ministry of Industry and Trade 2019). Fruit flies of the family Tephritidae are among the most destructive agricultural pests in the world (De Meyer et al. 2001). Because of their widespread agricultural impacts and rapid expansion, tephritid fruit flies are the subject of quarantine and control efforts, worldwide (White and Elson Harris 1992). There are about 450 genera and more than 4,300 described species within Tephritidae, making it one of the largest families within the order Diptera.

Anastrepha Schiner, *Bactrocera* Macquart, *Ceratitis* MacLeay, *Dacus* Fabritius and *Rhagoletis* Loew are the most economically important genera because many of their species are frugivorous. They infest almost every cultivated area and have wide bio-climatic adaptation potential (Bateman 1972). Thus, with the increasing movement of people and produce, they have the potential to invade other territories.

Sudan has enormous potential for horticultural production, over its wide range of climatic conditions and diverse ecosystems (Mahmoud 2011). There are several methods of fruit fly suppression, including the application of bait spray, male annihilation techniques, sterile insect techniques, and the use of biological control agents such as parasitoids and pathogens (White and Elson Harris 1992). The fungus *Metarhizium anisopliae*, a naturally occurring antagonist isolated from soil, is used in ICIPE to kill mature maggots and puparia of fruit flies (Ekesi and Billah 2006).

Social and environmental problems are currently associated with insecticide use for fruit fly control whether by aerial or ground applications on foliage against adults, or to soil for larvae or newly emerged adult control. Humans, domestic animals, and beneficial insects (natural enemies, pollinators etc.) can be affected by the

insecticides. The application of the same type of insecticide for a long period against particular insects may lead to lineages developing resistance. Certain pesticides provoke phytotoxic effects on various crops or varieties, especially under extreme climatic conditions in Sudan (Schmutterer 1969). Such problems have motivated the search for biological control alternatives, including entomopathogenic fungi.

This study aims at evaluating the efficacy of the fungus *M. anisopliae* against the pupae of *Bactrocera dorsalis* Hendel.

7.2 Materials and Methods

This experiment was carried out at the laboratory of the Plant Pathology Centre and the Biology Laboratory, Faculty of Agricultural Sciences, University of Gezira. The fungus, *M. anisopliae* Met. Sorokin, was isolated from a cadaver of a white grub, *Schizonycha* sp. (Coleoptera: Melolonthidae), on Kenana Sugarcane Farm by Elnour et al. (2009).

A laboratory assay was undertaken by applying four different concentrations (20×10^6, 10×10^6, 5×10^6 and 2.5×10^6) of *Metarhizium anisopliae* Sorokin on the pupae of *Bactrocera dorsalis* in order to test the potential to inhibit adult emergence.

7.2.1 Culture of the Fungus

Sabouraud Dextrose Agar (SDA, 32.5 g in 500 ml of distilled water) was dissolved by slightly heating on a hot plate and then autoclaved at 121 °C for 15 min under 15 psi. Next, the medium was cooled at room temperature and poured into 9-cm sterilized Petri dishes under aseptic conditions (Elnour et al. 2009). For isolation, a needle loop was used for intercepting the conidia on the cuticle of the insect host cadaver to inoculate the medium. After that, the culture was placed in an incubator at 27±0.5 °C for 3 weeks to get fungus ready for further multiplication and testing on other fruit flies.

7.2.2 Harvesting of Conidia

To harvest the spores, the surfactant emulsifier Tween 20% was added to Petri dishes containing the sporulating fungus. Conidia were suspended in the bathing media by stirring with a sterilised glass rod. The conidial suspension was discharged into a sterilised glass beaker, sieved through double-layer sterilised cloth in order to get rid of hyphal fragments. Other matter found within the conidia in the suspension was removed by washing, transferring the suspension to test tubes, for a 5 min centrifugation at 3000 rpm. This procedure was carried out in order to wet and disperse

the conidia. The supernatant or floating material was decanted and replaced by an aliquot of the washing solution. The conidia were then re-suspended using a vortex mixture, repeating this procedure four times. The concentration of the harvested conidia was then determined using a Neubauer ruled counting chamber (haemocytometer). The required spore concentrations were then prepared by dilution in sterilised distilled water (Hassan 1983; Elnour et al. 2009).

7.2.3 Application of the Fungus on Fruit Fly Pupae

Ripe and fallen fruits were collected from orchards, placed on ventilated bags and transferred to laboratory where they were distributed to small containers in equal numbers (5 fruits each). A sheath of sand had been previously placed at the bottom of the containers for pupation since the 3rd larval instar had begun springing. Fine cloth was added to the top of the containers for aeration purpose. After the larvae changed into pupae, the latter were placed into small vials (10 cm long, 5 cm internal diam.), with 10 pupae in each vial which contained a sheath of sand.

Four concentrations of the harvested conidia, 20×10^6, 10×10^6, 5×10^6 and 2.5×10^6 conidia/ml and the untreated control, were applied as treatments in 1 ml topically on the pupae of the invasive fruit fly *B. dorsalis*, using a micropipette. Treatments were arranged in a completely randomised design (CRD) with 3 replicates. Observations were taken daily on the pupal infestiation by the fungus and on adult emergence.

Data were recorded and the percentage mortality was taken, and subjected to Probit analysis using the Statistical Package for Social Sciences (SPSS). LC^{50} and LC^{90} were calculated in this experiment.

7.3 Results

Four different concentrations (20×10^6, 10×10^6, 5×10^6 and 2.5×10^6 conidia/ml) of *M. anisopliae*, were directly applied to the pupae of the oriental fruit fly, *B. dorsalis*, during this study. The four concentrations were effective in inhibiting the emergence of adults from the pupae, but in different degrees. Adult emergence from the pupae of *B. dorsalis* was inhibited by the tested concentrations of *M. anisopliae*, and the inhibition progressed as the concentration increased.

The relationship between dose concentrations and inhibition of emergence was in a direct proportion, as the inhibition of adult emergence increased with increasing doses of concentration, i.e. dose-dependent, while the log-dose Probit regression line was:

$$Y = 4.069X - 22.10.$$

Table 7.1 Inhibition of *Metarhizium anisopliae* of adult emergence of *Bactrocera dorsalis*

Concentration (10^6 conidia/ml)	No. of pupae	Observed response	Expected response	Probability
Untreated control	10	0	0	0
2.5	10	3	2.620	0.262
5	10	4	4.168	0.417
10	10	5	5.858	0.586
20	10	8	7.402	0.740

The mortality percentages of *B. dorsalis* pupae treated by *M. anisopliae* were 0%, 30%, 40%, 50% and 80% for doses: 0 (control), 2.5×10^6, 5×10^6, 10×10^6, 20×10^6, respectively.

The LC_{50} and LC_{90} values for the fungus were 10^6 and 18^6 conidia/ml, respectively. The heterogeneity factor ($\chi^2 = 0.576$), concentration, log concentration, tested response, corrected response, and tabulated Probit are shown in Table 7.1. The slope of the Ld-p line was stated in the equation above, and corresponded to 4.069.

7.4 Discussion

The entomopathogenic fungus *Metarhizium anisopliae* reproduces by mitotic spores (conidia). When these come into contact with the body of an insect host, they germinate and the emerging hyphae penetrate the cuticle. The fungus then develops inside the body, eventually killing the insect after a few days. The cuticle of the cadaver often becomes red. If the ambient humidity is high enough, a white mould then grows in the cadaver and soon turns green as conidia are produced (Freimoser et al. 2003).

The purpose of this study was to evaluate the efficacy of *M. anisopliae* on the pupae of the oriental fruit fly *B. dorsalis.* The relationship between dose concentrations and inhibition of adult emergence was in direct proportion: the inhibition of adult emergence increases with increasing dose concentration, in a dose-dependent relationship. The results of this study agreed with those of Musa (2013), who applied two concentrations, of 6.5×10^{10} conidia/ml and 4.3×10^8 conidia/ml, on *B. bassiana* and *M. anisopliae*, respectively, on two *Dacus* spp. The pure formulation of the fungi showed adult mortality between 42 and 90% and 29 and 77% in the two species, respectively.

7.5 Conclusion

Metarhizium anisopliae appeared suitable to control *B. dorsalis* as it affected the pupae with a relationship linking the applied dose concentration and the inhibition of adult emergence, in a direct proportion, in a classical dose-dependent relation-

ship. The LC_{50} and LC_{90} values for the fungus were 10×10^6 and 18×10^6 conidia/ml, respectively. *Metarhizium anisopliae* proved to be an effective bio-agent against the pupae of the oriental fruit fly and may be recommended to be used in suitable formulation for control.

Although the mentioned findings suggest the fungus can be incorporated in a fruit fly management strategy, field studies on the application of *M. anisopliae* on immature and adult stages of fruit flies are, however, required.

References

Bateman, M. A. (1972). *The Ecology of Fruit Flies. C.S. I. R., School of Biological Sciences*, University of Sydney, N. S .W. 2006, Australia.

De Meyer, M., et al. (2001). Taxonomic notes on the Afrotropical subgenera *Ceratitis* (Acroptromma) Bezzi and C. (Holpolophomiya) Bezzi (Diptera: Tephritidae). *Cimbebasia, 17*, 77–84.

Ekesi, S., & Billah, M. K. (2006). *A field guide to the management of economically important Tephritid fruit flies in Africa*. Nairobi: The International Centre of Insect Physiology and Ecology (ICIPE) Science Press.

Elnour, A. E., Ahmed, E. M., Rudwan, M. K., Yousif, F. M., & Bernard, V. (2009). Effects of temperature and substrate on vegetative growth of the entomopathogenic fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin. *Gezira Journal of Agricultural Sciences, 7*, 124–137.

Fatima, A. A., & Dawoud, H. D. (2017). *Mango cultivation and production in Sudan* (pp. 17–18). Ministry of Agriculture and Forestry, Agricultural Research Corporation, Horticulture Crop Research Centre.

Freimoser, F. M., Screen, S., Bagga, S., Hu, G., & St. Leger, R. G. (2003). EST analysis. *Microbiology, 149*, 239–247.

Hassan, A. E. M. (1983). *The interaction of entomopathogenic fungus, Metarhizium anisopliae (Sorokin) and the insecticide diflubenzuron on Manduca sexta (Johnson)*. PhD thesis, University of Bath, United Kingdom.

Mahmoud, M. E. E. (2011). *Population dynamic, mass trapping and molecular characterization of major fruit flies (Diptera: Tephritidae) in Khartoum, Kassala and Kordofan States (Sudan)*. PhD thesis, Department of Zoology, Faculty of Sciences, University of Khartoum, Sudan.

Ministry of Industry and Trade, Sudan. (2019). *Sudan trade point: mango*. www.tpsudan.gov.sd.

Musa, I. O. (2013). *Effects of NeemAzal 1% and two entomopathogenic fungi on some cucurbit fruit flies (Diptera: Tephritidae)*. PhD thesis, Faculty of Agriculture, Department of Crop Protection, University of Gezira.

Schmutterer, H. (1969). *Pests of crops in Northeast and Central Africa, with particular reference to the Sudan*. Stuttgart: Gustav Fisher Verlag. 257 pp.

White, I. M., & Elson Harris, M. M. (1992). *Fruit flies of economic significance: Their identification and bionomics* (601 pp). Wallingford: CAB International.