less susceptible variety released a more complex VOC blend than the other. Two VOCs, hexanal and β-caryophyllene, were found in the VOC profiles of maize and potatoes, and were tested for attractiveness in olfactometer assays. We are also testing the combination of these compounds in alginate beads containing EPN for attractiveness and biocidal effects under laboratory conditions. Using VOCs as attractants and EPN as biological agents represent a promising alternative to pesticides that remains to be evaluated in the field.

CONTRIBUTED PAPERS. Monday, 15:45 N-6

Do all *Xenorhabdus* and *Photorhabdus* bacteria protect nematode infected cadavers against scavengers?

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The Scavenger Deterrent Factor (SDF) activity of 30 *Xenorhabdus* isolates (26 species with one species having two strains and another species having three strains) and six *Photorhabdus* isolates (four subspecies of *P. luminescens*, one subspecies of *P. temperata*, and one subspecies of *P. asymbiotica*) was tested against the insect scavengers, *Gryllus bimaculatus* (cricket) and *Tapinoma madeirense* (ant) using agar plugs containing 3-day-old bacterial supernatants and 3-day-old *Xenorhabdus*- or *Photorhabdus*-killed insect larvae (*Galleria mellonella*). Results indicated that all *Photorhabdus* isolates tested had high SDF activity against both scavengers, whereas some of the *Xenorhabdus* species had deterrent activity against both scavengers, but others did not. For example, both the ants and crickets consumed the agar plugs with supernatants containing *X. beddingii*, *X. poinarii*, *X. ehlersii*, *X. japonica*, *X. hominickii*, *X. doucetiae*, *X. romanii*, *X. sapoensis*, *X.* sp. TS4, *X. koppenhoeferi*, and *X. bovienii* SS-2004 indicating no detectable SDF activity against the tested cricket and ant. Interestingly, the ants consumed agar plugs with *X. hominickii* and *X. ishibashii*, but the crickets did not. Thus, SDF plays a significant role in the survival of some EPNs and their bacterial symbionts in the insect cadaver in nature. However, it is not produced by all *Xenorhabdus* species, and the reasons for this lack of SDF activity against the tested cricket and ant species are not known.

CONTRIBUTED PAPERS. Monday, 16:00 N-7

Characteization of some entomopathogemic nematodes and fungi from the soil of Afghanistan

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A study was carried out to isolate and identify entomopathogenic nematodes and fungi from northern soil habitats of Badakhshan province, Afghanistan through 2017-2018. Here five isolates of nematodes and two isolates of fungi are reported. Two nematode isolates were from *Diploscapter* (Nematoda: Diploscapteridae) and also two species were from *Oscheius* (Nematoda: Rhabditida). Morphological studies with light microscopy and scanning electron microscopy, as well as molecular analyses using full-length small subunit rDNA gene of D2/D3, 18S and ITS genes has done. From fungi, the isolates were from *Metarhizium* (Ascomycota: Clavicipitaceae). The molecular characterisation as well class identification showed the isolates as *M. robertsii*. This is the first insight into diversity of these insect pathogens from Afghanistan.

CONTRIBUTED PAPERS. Monday, 16:15 N-8 STU

Identification of entomopathogenic nematodes in Central Anatolia with a comparison of two barcoding loci

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Entomopathogenic nematodes (EPNs) which belong to the families Steinernematidae and Heterorhabditidae and have a mutualistic association with bacteria that kill the host insect by septicemia and make the cadaver suitable for the development of the EPNs. The objective of this study is to identify EPNs isolated from agricultural fields of the Central Anatolia. The identification of EPNs which are adapted to climatic conditions of these areas is important for integrated pest management (IPM) programs in Turkey. Totally, 300 soil samples were collected and stored in 250 ml plastic containers. The nematodes were isolated from these samples with *Galleria mellonella* bait traps. Infective juveniles were collected with White traps and identified by sequencing of the ITS and mtCOI regions. EPNs identified in agricultural fields in Central Anatolia were *Heterorhabditis bacteriophora*, *H. marelatus*, *Steinernema* sp., *S. affine*, *S. bicornutum*, *S. carpocapsae*, *S. feltiae*, *S. litorale* and *S. weiseri*. This study provide both detailed information about EPN species and also potential biopesticide candidates for sustainable IPM programs of Turkey. In order to explore the diversity of EPNs in Turkish soils much more study should be required.

Coffee Break — Monday, 16:30-17:00, Foyer

CONTRIBUTED PAPERS — Monday, 17:00-19:00
VIRUS 1 — Auditorium 2

Virus Discovery and taxonomy

Chairs: Robert Harrison / Elisabeth Herniou

CONTRIBUTED PAPERS. Monday, 17:00 V-1

Divergence from the PDV paradigm in the repeated evolution of associations between mutualistic viruses and parasitoid wasps

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Some lineages of parasitoid wasps have evolved a remarkable strategy in their parasitism arsenal: they utilize symbiotic viruses to breach host defenses. The most well-known examples are Polydnaviruses (PDVs), which are found in three diverse clades of parasitoid wasps. Despite their independent evolution, PDV genomes share some key characteristics including wasp genome-integration and dispersal of viral genes into two separate components: proviral segments (containing virulence genes) and replication genes. The replication machinery for these viruses is not packaged into virions, thus the viruses and parasitoids are reliant on each other for reproduction. Non-PDV symbiotic viruses have been documented in other parasitoid wasp species, and their recent genomic characterization has shown that they diverge from the PDV paradigm. The genome

Isolation and Characterization Native Insect Pathogens from Soil of North Afghanistan

Mohammad Hussain Falahzadah[1,2], Javad Karimi[2*], Erahim Shokoohi[3], Gholamhossein Moravvej[2], Eustachio Tarasco[4]

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IOBC-WPRS

Abstract

In a survey of entomopathogenic nematodes (EPN) and entomopathogenic fungi (EPF) in north Afghanistan, we identified two EPN which belong to genus *Oscheius* and *Diploscapter* and two species of EPF belong to genus *Metarhizium*. The conidia suspension was applied against subterranean termite, *Anacanthotermes vagans*.

Introduction

Entomopathogenic nematodes: A huge number of entomogenous nematoeds have been reported from insects. These vectors entomopathogenic bacteria inside the hemocoel of a suitable host (lacey, 2017) (Fig. 1).

Entomopathogenic fungi: 100 genera with approximately 750 species, reported from different insects and living in diverse habitats including fresh and soil surface, many of them have potential in pest management (Azmi, et al., 2011; Chandler, 2017) (Fig 2).


Fig. 1. Life cycle of entomopathogenic nematodes (Ffrench-Constant, 2003).

Fig. 2. Shows diverse hosts of EPF

Materials and methods

- The soil samples were collected from northern province of Afghanistan (Fig. 3).
- Soil samples were baited with *Galleria mellonella* larvae. The pathogens characterized based on molecular and classical data (Fig. 4).

Fig. 4. Showing PCR of EPN and EPF with electrophoresis

Evaluation of pathogenicity against termites at laboratory assay: three strains including *Metarhizium robertsii* (Afghan isolate), *Beauveria bassiana* and *Beauveria varroa* were used for assay against *Anacanthotermes vagans*. 600µl suspension of each fungal concentration were poured inside the sterile Petri dish with sterile filter paper. Mortality was observed after 24h (Fig. 5.).

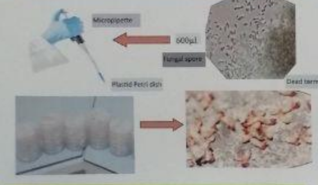

Fig. 5. Fungal suspension were poured inside the sterile plastic Petri dish with sterile filter paper

Evaluation of pathogenicity against termites at semi field assay

- two strains including *Metarhizium robertsii* and *Beauveria bassi* were used.
- prepared plastic container
- 2.5ml fungal suspension sprayed (Fig. 6).

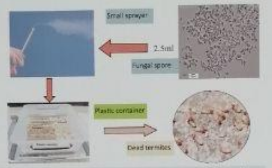

Fig. 6. Spray fungal suspension with small sprayer inside the plastic container

Result

Entomopathogenic nematodes:

- EPN identified as *Oscheius tipulae* and *Diploscapter coronatus* based on morphological data and molecular analysis(Fig 7-8).
- For molecular identification ITS, 18S and D2/D3 were used (Fig. 9-10).

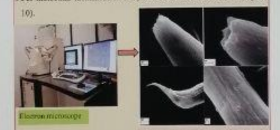

Fig. 7. *Oscheius tipulae* scanning electron micrographs . A: Annules; B: Head; C: Vulva region; D: anus.

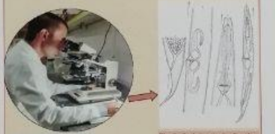

Fig. 8. *Oscheius tipulae* female. A: Tail region. B: Vulva region. C: Pharyngeal region. D: Entire body.

Entomopathogenic Fungi: The EPF species are identified as *M. robertsii* and *M. anisopliae* based on morphological data and molecular analysis. For molecular identification ITS4-5 region was used (Fig. 11).

Insecticidal activity of *Metarhizium* and *Beauveria* under the laboratory condition: In the laboratory, all isolates caused significant different mortality rate [(F=104.08, df= (2, 30), p<0001)] in comparison to control groups (Table 1).

Pathogenicity of *M. robertsii* and *B. bassianie* under the semi-field condition: both isolates have significant mortality [(F= 64.22, df= (1, 20), p<0001)]. The results showed that *M. robertsii* was had high mortality (Fig. 12). After 6 days conidia were grown (Fig. 13).


Fig. 13. Mycosis development on the surface body of termite after 6 days. A= *Metarhizium* B= *Beauveria*

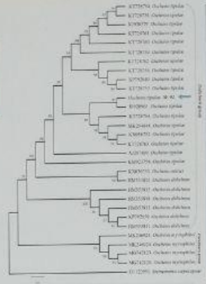
Fig. 9. Phylogenetic relationships of *Oscheius tipulae* and other closely related Rhabditidae with *Steinernema carpocapsae* as the outgroup in MEGA7 tree based on analysis of ITS region sequence data.

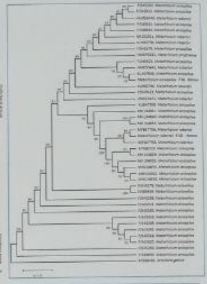
Fig. 11. Phylogenetic analysis of new isolated strains of *M. robertsii* and *M. anisopliae* to closely related species/ strains based on ITS gene sequences using maximum likelihood.

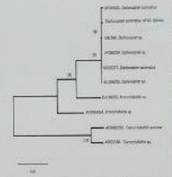
Fig. 10. MEGA7 Phylogenetic tree of *Diploscapter coronatus*. Posterior probabilities greater than 50% are given on appropriate clades. Nematode species, GenBank accession numbers and locations are listed for each taxon.

Table 1. Pathogenicity of three entomopathogenic fungi strains from *B. bassiana*, *B. varroa* and *M. robertsii* on termite, *Anacanthotermes vagans* under the laboratory condition

Days after treatment	2	4	6	8	10
Fungal species [Concentration (conidia/ml^{-1})]	% Mortality (± SE)				
B. bassiana					
Concentrations conidia/ml^{-1}					
0.05% Tween-80	0.0 ± 0.0	0.0 ± 0.0	3.33 ± 3.3	6.7 ± 3.3	6.7 ± 3.3
1× 10^{5} conidia ml^{-1}	3.3 ± 3.3	13 ± 6.7	43.3 ± 6.7	63 ± 3.3	100 ± 0.0
1× 10^{6}	3.3 ± 3.3	20 ± 5.7	63.2 ± 3.3	90 ± 2.7	100 ± 0
1× 10^{7}	6.7 ± 3.3	23 ± 8.8	70 ± 11.5	100 ± 0.0	100 ± 0.0
1× 10^{8}	12.4 ± 3.3	40 ± 5.7	100 ± 0.0	100 ± 0.0	100 ± 0.0
B. varroa					
0.05% Tween-80	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 3.3	6.67 ± 3.3	6.67 ± 3.3
1× 10^{5} conidia ml^{-1}	3.3 ± 3.3	3.3 ± 3.3	3.3 ± 3.3	10.0 ± 3.3	23.3 ± 3.3
1× 10^{6}	3.3 ± 3.3	3.3 ± 3.3	6.7 ± 3.3	13.3 ± 3.3	26.7 ± 3.3
1× 10^{7}	3.3 ± 3.3	3.3 ± 3.3	10.0 ± 5.7	23.3 ± 3.3	30.0 ± 0.0
1× 10^{8}	0.0 ± 0.0	10 ± 3.3	20.0 ± 5.7	30.0 ± 7.7	40.0 ± 7.7
M. robertsii					
0.05% Tween-80	3.0 ± 3.0	10 ± 0.0	10.0 ± 0.0	10 ± 0.0	10.0 ± 0.0
1× 10^{5} conidia ml^{-1}	10 ± 6.7	40 ± 10	100 ± 0.0	100 ± 0.0	100 ± 0.0
1× 10^{6}	13.3 ± 3.3	56 ± 10	100 ± 0.0	100 ± 0.0	100 ± 0.0
1× 10^{7}	23.3 ± 8.8	76 ± 8.8	100 ± 0.0	100 ± 0.0	100 ± 0.0
1× 10^{8}	53.3 ± 12	100 ± 8.8	100 ± 0.0	100 ± 0.0	100 ± 0.0

Insecticidal effects of fungal species against *A. vagans* at four different conidial concentrations. Values expressed as the mean of three replicates. Different upper case letter indicates the significant difference (F= 27.21, df= 30, P<0.0001) at Tukey HSD.

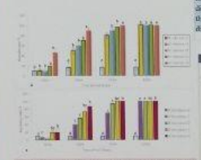
Fig. 12. Virulence of difference concentrations (c0, c1, c2, c3, c4=concentration) of *M. robertsii* (a) and *B. bassiana* (b) on the termite, *Anacanthotermes vagans* at 1-12 days (DAA: Days after applied). Bars indicate standard deviation of mean. Different letter indicates significant differences between effect of species and concentrations according Tukey HSD.

Fig. 3. Map of Afghanistan

Conclusion

- Afghanistan has a rich fauna and flora biodiversity resource.
- Unfortunately, as a result of war does not identify beneficial organism.
- The present study we identified two species of entomopathogenic nematode and two species of entomopathogenic fungi from the soil of northern Afghanistan.

Reference

1. Azmi, W. A., Sulaiman, Z. A., Ishak, I., Kin, P. K., Eam Lin, G. L., and Addis, S. N. K. 2011. Virulence evaluation of entomopathogenic fungi to subterranean termites, *Globitermes sulphureus* (Insecta: isoptera). *Malaysian Journal of Microbiology, 12: 492-497.*

2. Chandler, D. 2017. Basic and applied research on entomopathogenic fungi. In: L. A. Lacey, 2nd (ed.), *Microbial Control of Insect and Mite Pests. Academic Press is an imprint of Elsevier, PP 70-83.*

3. Lacey, L. A. 2017. Entomopathogens Used as Microbial Control Agents. In: L. A. Lacey, 2nd (ed.), *Microbial Control of Insect and Mite Pests. Academic Press is an imprint of Elsevier. PP 3-9.*