EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

ENTOMOLOGY

A

ISSN
1687-8809

WWW.EAJBS.EG.NET

Vol. 10 No. 3 (2017)

Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

Entomology Journal publishes original research papers and reviews from any entomological discipline or from directly allied fields in ecology, behavioral biology, physiology, biochemistry, development, genetics, systematics, morphology, evolution, control of insects, arachnids, and general entomology.

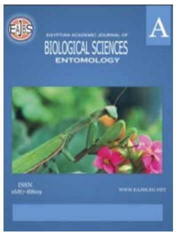
www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 10(3)pp: 29- 34 (2017)*

Egypt. Acad. J. Biolog. Sci., 10(3): 29–34 (2017)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
www.eajbs.eg.net



Bioactivity of Trichoderma (6-Pentyl α-pyrone)against *Tetranychus urticae* Koch (Acari: Tetranychidae)

Salwa, Sholla, M. E [1] and Metwally R. Kottb[2]
1- Plant Protection Research Institute (PPRI) Giza – Egypt
2- Department of Botany, Faculty of Science, Suez Canal University, Ismailia, Egypt.

ARTICLE INFO
Article History
Received: 20/3/2017
Accepted: 29/4/2017

Keywords:
Trichoderma
Volatiles
Fungi
Mites
Biocontrol

ABSTRACT

Several Trichoderma species are used as biocontrol agents due to their high potentiality to produce a wide array of bioactive secondary metabolites; (some of these metabolites are low molecular weight volatiles). *Trichoderma asperellum* was isolated from the rhizosphere of Zea mays at Ismailia, which showed high antifungal and nematoicidal activity from our preliminary experiments. Bioactivity of *T. asperellum* against mite *Tetranychus urticae* koch was tested. Data showed that high mortality was observed after exposure time (48 hrs.) and reached 100%. The major volatile compound emitted from *T. asperellum* was identified to be 6-pentyl- α-pyrone (6PP), with rate 450 ng/µl. This high level of 6PP motivated us to apply different concentrations of pure compound (0.125, 0.25, 0.50 and 1mM of 6PP (sigma) to find out their effect.

INTRODUCTION

The two spotted spider mite *Tetranychus urticae* Koch is one of the most important pests of many horticultural and field crops, including fruits, cotton, vegetables and ornamentals. This mite has been reported to attack about 1200 species of plants, of which more than 150 are economically significant (Zhang and Schlyter, 2004), currently, the cost of their use to counter spider mite damage exceeds USD $ 1 billion per year in the European Union due to the excessive use of pesticides and the associated problems of resistance and environmental pollution.

Extensive use of chemicals can cause environmental hazards and induce resistance. Microorganisms are major candidates, of which entomopathogenic fungi are most promising control agents, since they invade the insect host by contact. Biological control with pathogenic fungi might provide long-lasting insect control without damage to the environment or non-target organisms (Hokkanen and Lynch, 1995; Kessler *et al.*, 2004). Much of this research has been focused on hyphomycetes: *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorokin, two fungi believed to be well adapted to the soil ecosystem (Keller and Zimmerman, 1989).

Entomologists have been pioneers of chemical ecology so it is not surprising that the best known cases of Volatile Organic Compounds VOCs signalling involve chemical communication with an emphasis on microbial gas phase molecules. Many fungal volatiles, particularly eight carbon alcohols and ketones, act as semi chemicals and function in insect attraction and deterrence (Dowd and Bartelt, 1991; Pierce *et al.*, 1991; Nilssen, 1998; Ramoni *et al.*, 2001; Luntz, 2003).

This study aimed to reach an integrated control for spider mite, T. urticae on plants by means of safe Trichoderma volatiles 6-PP.

MATERIALS AND METHODS

Two spotted spider mite culture

The experimental mites were obtained from castor leaves *Ricinus communis* L. from the farm of Agricultural Cairo University. *T. urticae* were reared in the laboratory on fresh leaves of Acalypha, *Acalypha marginata* for one generation under laboratory condition before the experiments to establish the mite.

Simply, by collecting severely infested castor leaves *Ricinus communis* by egg and larvae of this pest. Individuals of 20 eggs *T. urticae* were transferred to Acalypha, leaf discs (2×5cm diameter) placed on cotton bed in Phil dishes (20×15 cm). The cotton bed was kept wet by soaking with water twice daily, so that the discs remained fresh.

Trichoderma species used in this study were isolated from different soils from Egypt during the period 2005 – 2006 (Kottb, 2008). Trichoderma was identified at a molecular level according to Carbone & Kohn (1999), by amplification of the ITS regions, including ITS-1, ITS-2 and the 5.8S rDNA gene (primers V9G and LS266) and the Elongation factor (primers EF1-728f and TEF1-LLErev). This species was deposited at the centraal bureau voor schimmel cultures (CBS) under accession number CBS137093.

Bio-activity of Trichoderma VOCs against Tetranychus urticae

Insecticidal activity of *T. asperellum* VOCs was tested (in vitro assay) against the two-spotted spider mites "*Tetranychus urticae*"in 15 cm petri dish. One disc (0.5 cm diameter) of 7 days old Trichoderma culture was inoculated into 7 cm petri dish containing 10 ml broth medium (Bonnarme *et al*., 1997), composed of glucose 30 g l; $NaNO_3$ 2 g; KH_2PO41 g l; yeast extract 1 g; pepton2 g; KCl 0.5 g; $MgSO_4{\cdot}7H_2O$ 0.5 g; $CaC_{l2}{\cdot}6H_2O$ 8 mg; $ZnSO_4{\cdot}7H2O$ 1 mg; $FeSO_4{\cdot}7H_2O$ 10 mg. One plate containing non-inoculated medium was used as control, while the other plate containing the medium inoculated with Trichoderma. The plates were placed in the same plate 3 cm apart from the *Ricinus communis* plant leaves on it 10 individuals of mites. The percentage of mortality was recorded after 2, 3 and 4 days.

Insecticidal activity of different concentrations of pure 6PP

By applying (0.125, 0.25, 0.5 and 1 mM) of 6PP (sigma) soluble in sterile distilled water directly apply on sponge piece till saturation. The effect of the biologically equivalent concentration relevant to that produced by Trichoderma on the mites' vitality. The application of 6PP was directly in one set and by fumigation percentage of mortality was recorded after 2 and 3 days in three replicates. The experiment was examined daily and results were recorded. The values of LC50 and slop were determined by computerized probity analysis program.

RESULTS AND DISCUSSION

The head space volatiles emitted from *T. asperellum* were analyzed. This experiment carried out as mentioned in (Kottb *et al*., 2015). The dynamic of the volatile emission was recorded throughout 10 days with 24h intervals. The highest level of the bioactive volatile compound 6-pentyl-α-pyrone (6PP) was recorded by GC-MS chromatogram at RT 20.9**.** The identification of this compound has been confirmed by injection of the authentic pure compound of (Sigma).

Since 6PP is the major volatile compound emitted by *T. asperellum*, the effects of this compound were tested at different concentrations. Serial dilutions 0.125, 0.25, 0.5 and 1 mM of pure 6PP (which is biologically equivalent to the amount emitted from *T. asperellum* showed highly inhibitory effect on. The percentage of mortality significantly increased scored as concentration increased and the mortality after the application of 1 mM of 6PP and also after exposure to *T. asperellum* the percentage of mortality reached to 100%.

Data in Table (1) and illustrated in Figs. (1& 2) revealed that concentration of 1mMol of *T. asperellum* was able to kill 90% after one day. By contrast spider in the control treatment mortality was achieved by 10%. Mortality increased as the concentration increased. The LC_{50} and LC_{95} were calculated as 0.33 and 1.21 mMol. Thus concentration of 1.00 mMol of 6PP0 was more effective against two spotted spider mite.

Table 1: Effect of *T. asperellum* (6pp) against adult stage of *T. urticae.*

Concentrations (mMol)	No. of adult	No of dead	Mortality (%)
1.00	30	27	90
0.5	30	24	80
0.25	30	12	40
0.125	30	6	20
Control	30	3	10

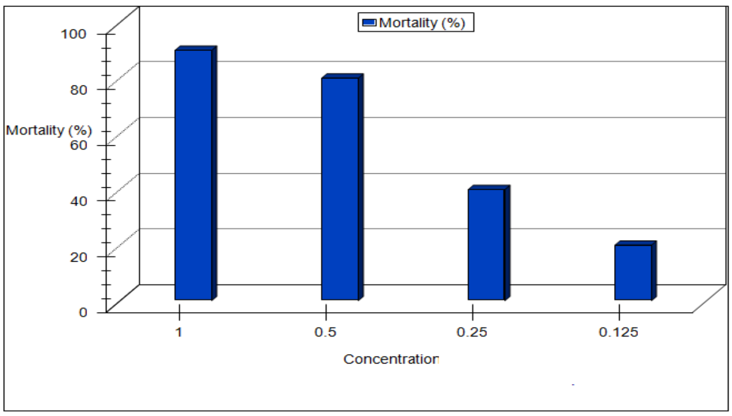


Fig. 1: Effect of *T. asperellum* (6PP) against adult stage of *T. urticae.*

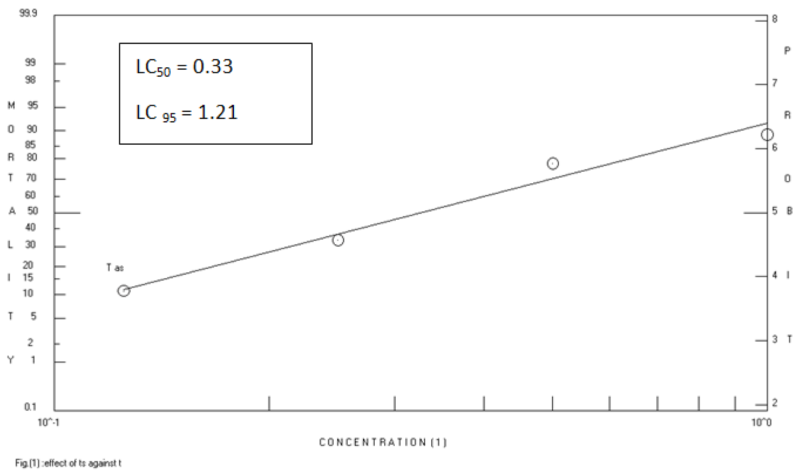


Fig. 2: Regression line for the relative toxicity of *T. asperellum* (6pp) against adult stage of *T. urticae*.

The present investigation was carried out to elucidate the relative virulence of

(6pp) against two spotted spider mite. Fungal virulence has been used as the most important criterion for selecting fungal species or isolate for microbial control of agricultural pests (Lai *et al*., 1982; Zoberi and Grace, 1990; Wells *et al.,* 1995; Almeida *et al.,* 1997). This has not resulted in successful field trials of fungi against some agricultural pests (Zoberi, 1995; Sajap *et al*., 1997). Pathogenicity test of the isolated entomopathogenic fungi against some economic insect pests showed that some isolates of fungi had a wide range of hosts. The entomopathogenic fungi could be considered the most promising candidate as biocontrol of many insect pests.

Our study reported that *T. asperellum* (6pp) was more effective against *T. urticae* as indicated by low value of LC_{50}.

In conclusion, from the aforementioned results it could be concluded that biological control with pathogenic fungi is a promising alternative to chemical control against the agricultural pests all over the world (Wells *et al.,* 1995; Grace, 1997; Milner *et al*., 1998). Extensive use of chemicals can cause environmental hazards and induce resistance. Biological control with pathogenic fungi might provide long-lasting insect control without damage to the environment or non-target organisms (Hokkanen and Lynch, 1995). The Deuteromycetes, *M. anisopliae* and *B. bassiana* are common soil borne entomopathogenic fungi that occurs worldwide (McCoy *et al.,* 1988). Deuteromycete entomogenous fungi, *B. bassiana* and *M. anisopliae*, have major advantages for insect biological control. The primary reasons or interest in these fungi (McCoy *et al*., 1988; Fuxa, 1997) are their entry by contact, replication in target insects, safety to non-target organisms (Hokkanen and Lynch, 1995), numerous strains (St. Leger *et al.,* 1992), and *in vitro* mass-culture (Jackson *et al.,* 2000). They are a promising alternative to chemical control for some agricultural pests (Grace, 1997).

For these reasons, an inoculative augmentation approach may be promising for insect control with fungi (Fuxa, 1987; 1995). A fungus introduced through attractant-baited traps has the potential to replicate and spread to produce epizootics in insect population (Fuxa *et al*., 1998).

REFERENCES

Almeida, J.E.M., Alves, S.B. and Pereira, R.M. (1997). Selection of *Beauveria* spp. isolates for control of the termite *Heterotermes tenuis* (Hagen, 1858). J. Appl. Entomol. 121: 539–543.

Bonnarme, P., Djian, A., Latrasse, A., Féron, G., Giniès, C. and Durand, A.. (1997). Production of 6-pentyl-a-pyroneby *Trichoderma* sp. from vegetable oils. *J. Biotechnol*. 56: 143–150. doi:10.1016/S0168-1656 (97)00108-9

Carbone, M. and Kohn. S. (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia, 85: 415—427.

Dowd, P.F. and Bartelt, R.J. (1991). Host-derived volatiles as attractants and pheromone synergists for dried fruit beetle, Carpophilushemipterus. J. Chem. Ecol., 17: 285–308.Fuxa, J.R. (1987). Ecological considerations for the use of entomopathogens in IPM. Annu. Rev. Entomol., 32: 225–251.

Fuxa, J.R., (1987). Ecological considerations for the use of entomopathogens in IPM. Annu. Rev. Entomol. 32, 225–251.

Fuxa, J.R. (1995). Ecological factors critical to the exploitation of entomopathogens in pest control. In: Hall, F.R., Barry, J.W. (Eds.), Biorational Pest Control Agents: Formulation and Delivery. American Chemical Society, Washington, DC, pp. 42–67.

Fuxa, J.R. (1997). Microbial control of insects: status and prospects for IPM. In: Upadhyay, R.K., Mukerji, K.G., Rajak, R.L. (Eds.), IPM System in Agriculture. Biocontrol in Emerging Biotechnology, vol. 2. Aditya Books, New Delhi, pp. 57–104.

Fuxa, J.R., Ayyappath, R. and Goyer, R.A. (1998). Pathogens and Microbial Control of North American Forest Insect Pests. USDAForest Service, Forest Health Technology Enterprise Team, Morgantown, WV.

Grace, J.K., (1997). Biological control strategies for suppression of termites. J. Agric. Entomol. 14: 281–289.

Hokkanen, H. and Lynch, J.M. (1995). Biological Control: Benefits and Risks. Cambridge University Press, Cambridge, UK.

Jackson, T.A., Alves, S.B. and Pereira, R.M. (2000). Success in biological control of soil-dwelling insects by pathogens and nematodes. In: Gurr, G., Wratten, S.D. (Eds.), Biological Control: Measures of Success. Kluwer Academic Publishers, Dordrecht, pp. 271–296.

Keller, S. and Zimmerman, G. (1989). Mycopathogens of soil insects. In: Wilding, N., Collins, N.M., Hammond, P.M., Webber, J.F. (Eds.), Insect– Fungus Interactions. Academic Press, London, UK, pp. 240–270.

Kessler, P., Enkerli, J., Schweizer, C. and Keller, S. (2004). Survival of *Beauveria brongniartii* in the soil after application as a biocontrol agent against the European cockchafer, *Melolontha melolontha*. Biocontrol, 49: 563–581.

Kottb, MR. (2008). Survey and characterization of *Trichoderma* and Gliocladium species and specification of their biocontrol ability. M.Sc. thesis (Microbiology), Suez Canal University, Ismailia, Egypt.

Kottb, M., Gigolashvili, T., Großkinsky, D. and Piechulla B. (2015). Trichoderma volatiles effecting Arabidopsis: from inhibition to protection against phytopathogenic fungi Frontiers in Microbiology, 6, Article 995.

Lai, P.Y., Tamashiro, M. and Fuji, J.K. (1982). Pathogenecity of six strains of entomogenous fungi for *Coptotermes formosanus*. J. Invertebr. Pathol. 39: 1–5.

Luntz, A.J. (2003). Arthropod semiochemicals: mosquitoes, midges and sealice. Biochem.Soc.Trans., 31: 128–133.

McCoy, C.W., Samson, R.A. and Boucias, D.G. (1988). Entomogenous fungi. In: Ignoffo, C. and Mandava N.B. (eds) CRC Handbook of natural Pesticides, Vol. 5. Microbial Insecticides, part A: Entomogenous Protozoa and fungi. CRC Press, Boca Raton, Florida, pp.151-234.

Milner, R.J., Staples, J.A., Hartley, T.R., Lutton, G.G., Driver, F. and Watson, J.A.L. (1998). Occurrence of *Metarhizium anisopliae* in nests and feeding sites of Australian termites. Mycol. Res., 102: 216–220.

Nilssen, A.C. (1998). Effect of 1-octen-3-ol in field trapping *Aedes* spp. (Dipt., Culicidae) and *Hybomitra* spp. (Dipt., Tabanidae) in subarctic Norway. J. Appl. Entomol., 122: 465–468.

Pierce, A.M., Pierce, H.D., Oehlschlager, A.C. and Borden, J.H. (1991). 1-Octen-3-ol, attractive semiochemical for foreign grain beetle, *Ahasverusadevna* (Waltl) (Coleoptera: Cucujidae). *J. Chem. Ecol.*, 3: 567–579.

Ramoni, R., Vincent, F., Grolli, S., Conti, V., Malosse, C., Boyer, F., Nagnan-Le., Meillour, P., Spinelli, S., Cambillau, C. and Tegoni, M. (2001). The insect attractant 1-octen-3-ol is the natural ligand of bovine odorant binding protein. J. Biol. Chem., 276: 7150.

Sajap, A.S., Atim, A.B. and Hussin, H. (1997). Isolation of *Conidiobolus coronatus* (Zygomycetes: Entomophthorales) from soil and its effect on *Coptotermes* *curvignathus* (Isoptera: Rhinotermithidae). Sociobiology, 30: 257–262.

St. Leger, R.J., Frank, D., Robert, W. and Staples, R.C. (1992). Molecular cloning and regulatory analysis of the cuticle-degrading-protease structural gene from the entomopathogenic fungus *Metarhizium anisopliae*. Eur. J. Biochem., 204: 991–1001.

Wells, J.D., Fuxa, J.R. and Henderson, G. (1995). Virulence of four fungal pathogens to *Coptotermes formosanus* (Isoptera: Rhinotermitidae). J. Entomol. Sci., 30: 208–215.

Zoberi, M.H. and Grace, J.K. (1990). Isolation of the pathogen *Beauveria bassiana* from *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Sociobiology. 16(3): 289-296.

Zoberi, M.H. (1995). *Metarhizium anisopliae*, a fungal pathogen of *Reticulitermes flavipes* (Isopatera: Rhinotermitidae). Mycologia, 87: 354–359.

Zhang, Q.H. and Schlyter, F. (2004). Olfactory recognition and behavioural avoidance of angiosperm nonhost volatiles by conifer-inhabiting bark beetles. Agric. and Forest Entomol., 6(1): 1–20.

ARABIC SUMMERY

النشاط الحيوي لفطر الترايكودرما ضد اكاروس العنكبوت الأحمر

سلوى محمود السعيد شعلة[1]، متولى قطب[2]

١- معهد بحوث وقاية النباتات – مركز البحوث الزراعية.
٢- قسم النبات - كلية العلوم جامعة قناة السويس – الإسماعيلية – مصر.

يعتبر فطر الترايكودرما عنصر هام من عناصر المكافحة البيولوجية وذلك لانتاجه نواتج الهدم الثانوية النشطة. تم عزل فطر التريكودرما من منطقة الجذور لنباتات الذرة الشامية المنزرعة في محافظة الإسماعيلية. اتضح من الدراسة أن لهذه العزلة نشاط عالي ضد العديد من الفطريات الممرضة للنبات بالإضافة إلى نشاطها ضد النيماتودا المسببة لتعقد الجذور.

تم تقييم النشاط الحيوي لهذه النواتج ضد اكاروس العنكبوت الأحمر ذو البقعتين تحت الظروف المعملية. اتضح من الدراسة للفطر فعالية عالية ضد اكاروس العنكبوت الحمر بعد ٢٤ ساعة من المعاملة حيث أدت إلى حدوث موت بنسبة ١٠٠% فى الحيوانات الكاملة من الاكاروس. تم عزل وتعريف المواد الطيارة المنبعثة من الفطر ووجد انها 6-pentyl- α-pyrone (6PP) بمعدل 450 نانوجرام للميكرولتر. تم استخدام تركيزات مختلفة من الفطر ووجد انه مع زيادة التركيزات يزيد نسب الموت للاكاروس.