

Insecticidal activity of bio-oils and biochar as pyrolysis products and their combination with microbial agents against *Agrotis ipsilon* (Lepidoptera: Noctuidae)

Atef M.M. Sayed^{1*}, Robert W. Behle², Kari Tiilikkala³ and Steven F. Vaughn²

¹Agricultural Research Center, Plant Protection Research Institute, Biological Control Research Department, Giza, Egypt

²United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Crop Bioprotection Research Unit, Peoria, Illinois, USA

³MTT Agrifood Research Finland, Natural Resources Institute (Luke), 31600 Jokioinen, Finland

*Corresponding author: atef.mahmoud1@gmail.com

Received: 23 August 2017

Accepted: 3 January 2018

SUMMARY

Pyrolysis technology for producing biochar and bio-oils can be used as a potential alternative to make biopesticides, which are urgently needed in integrated pest management (IPM). Insecticidal activity of three components of bio-oils: aqueous, organic and their mixture, was evaluated individually and with three different entomopathogens: the baculovirus *Agrotis ipsilon* nucleopolyhedrovirus (*AgipMNPV*), bacterium *Bacillus thuringiensis* var. *kurstaki* (*Bt*) and fungus *Beauveria bassiana* (*Bb*) against black cutworm, *Agrotis ipsilon* (Hufnagel). The effect of alkaline conditions of spray-dried biochar was studied simultaneously with the microbial pathogens. Our bioassay results indicated that the organic bio-oil phase was the most active, causing 100% mortality after 24 h, when the median lethal toxicity values LC_{50s} (mg/ml) were found. However, the bio-oil fractions applied alone tended to cause higher mortality of the exposed larvae than did either mix with the microbial agents. Also, the results revealed that maximum mortalities were found in spray-dried formulations made with biochar at pH 7.1. It was concluded that pyrolysis oils are effective insecticides, and biochar could be a useful additive in production and formulation of biopesticides. This interesting finding further promotes the use of pyrolysis bio-oils and biochar compounds as eco-friendly alternatives to replace conventional pesticides.

Keywords: Pyrolysis products; Microbial agents; Insecticidal activity; *Agrotis ipsilon*

INTRODUCTION

There is now more than ever an urgent need to develop pest control methods which are environmentally safe and economically sustainable. Several studies have suggested some potential in biological alternatives to chemicals for cutworm control, such as parasitoids, entomopathogens, and nematodes (Ruiz de Escudero et al., 2014; Yan et al., 2014; Wraight et al., 2010). Some studies have suggested also that pyrolysis products, i.e. bio-oil and biochar, could be used as environmentally friendly biocontrol agents (Tiilikkala et al., 2011; Hou et al., 2015).

Pyrolysis is the thermal decomposition process used to convert agricultural residues, wood wastes and even municipal solid waste into energy, fuels, and value-added chemicals such as fertilizers (Czernik & Bridgwater, 2004). By heating waste materials in the absence of oxygen, biomass is converted into gas, liquid, and solid fractions (Sensoz et al., 2006).

Bio-oils, also known as pyrolysis oils, pyrolysis liquids, wood vinegar, pyrolygneous acid and others, are usually dark brown, free-flowing liquids having an acrid or a distinctively smoky odor (Czernik & Bridgwater, 2004). Bio-oils are multi-component mixtures comprised of different size molecules derived primarily from depolymerisation and fragmentation reactions of three key biomass building blocks: hemicellulose, cellulose and lignin (Lu et al., 2009; Fagernäs et al., 2012). Insecticidal activity has been reported for bio-oils produced by pyrolysis of tobacco leaves (Booker et al., 2010a,b), coffee grounds (Bedmutha et al., 2011), cellulose, hemicellulose, and lignin (Hossain et al., 2013), straw of canola, *Brassica* spp., (Suqi et al., 2014), residues of greenhouse tomato plants (Cáceres et al., 2015). Pyrolysis oils are known to be biodegradable (Hagner, 2013; Campisi et al., 2016).

Biochar is the carbonaceous solid residue produced by pyrolysis of biomass materials (Sharma et al., 2004; Laird et al., 2009). Biochar amendments to soils have aroused much interest for having a potential for climate change mitigation, soil improvement and increased crop productivity (Lehmann et al., 2011; Hou et al., 2015). However, little attention had been focused on the influence of biochar as a biopesticide for pest management. Insecticidal impacts of biochar are not well known but it has been proved that biochar amendment to soils impairs the developmental and reproductive performances of a major rice pest, the rice brown planthopper, *Nilaparvata lugens* (Hou et al., 2015).

Research of microbial pathogens of insects has increased considerably in recent years, offering an attractive environmental-friendly alternative or

supplement to chemical pesticides, the latter being potentially hazardous to health and the environment. The importance of biopesticides is expected to grow in the future (Chandler et al., 2011; Lacey et al., 2015). In particular, bacterial strains of *Bacillus thuringiensis* (*Bt*), entomopathogenic fungi (e.g. *Beauveria bassiana*), and numerous species of baculoviruses (Lacey & Kaya, 2007) are promising components for integrated pest management programs (IPM) (Gupta & Dikshit, 2010). However, these biocontrol agents are reported to be sensitive to environmental degradation, which leads to inconsistent and poor efficacy in the field. Bio-oils and biochar may be suitable to protect microbial pathogens from adverse environmental factors, and maximize application efficiency, facilitate leaf coverage, improve adhesion to plants, and provide UV protection.

As yet, there is no information available on combinations of bio-oils with insect microbial pathogens. Mixing microbial agents with pyrolysis products may produce synergistic, additive or antagonistic effects relative to insect control.

Another important issue that should be addressed is the determination of alkaline conditions of spray-dried biochar combined with three different entomopathogens: a baculovirus (*AgipMNPV*), bacterium (*Bt*) and fungus (*Bb*). In our previous research (Sayed & Behle, 2017b), differences in mixing activity were observed that may be related to the pH of biochar. The result was somewhat unexpected and needed to be studied before biochar is used for production and formulation of biopesticides. In another publication, biochar appeared to stimulate soil microbial activity and increased fungi abundance and functioning (Warnock et al., 2007). Bacterial abundance also increased with biochar addition, especially at a higher dosage, and in particular for *Bacillus* and *Pedomicrobium*, which were linked to alteration of physicochemical soil properties (Yao et al., 2017).

The larva of *Agrotis ipsilon* (Hufnagel) (Lepidoptera, Noctuidae), commonly known as cutworm, is a serious polyphagous pest of different economic vegetables, field crops and golf course grasses around the world (El-Salamouny et al., 2003; Hong & Williamson, 2004). However, adequate control of this pest with chemical pesticides is difficult to achieve. Hence, alternative and more consumer-friendly and environmentally safe bioproducts are needed for the control of cutworms. Several studies have suggested some potential of biological alternatives to chemicals for cutworm control (Gokce et al., 2013; Wraight et al., 2010; Caballero et al., 1993).

The objective of the present study was to determine the efficacy of pyrolysis bio-oil fractions as a new

alternative pesticide against black cutworm *A. ipsilon* (Hufnagel). Another aim was to evaluate the toxicity of mixtures of biomass pyrolysis bio-oils with three different entomopathogens: the baculovirus *Agrotis ipsilon* nucleopolyhedrovirus (*AgipMNPV*), bacterium *Bacillus thuringiensis* var. *kurstaki* (*Bt*) and fungus *Beauveria bassiana* (*Bb*). The third objective was to study the impact of alkaline conditions of spray-dried biochar when combined with the microbial pathogens.

MATERIALS AND METHODS

Insect colony, Agrotis ipsilon (Hufnagel)

All assays used neonates of black cutworm, *A. ipsilon*, from colonies maintained on artificial diets (Southland media, Southland Products INC., USA) at $25 \pm 2^\circ\text{C}$, $55 \pm 10\%$ RH, and a photoperiod of 16:8 (L:D) h, in the National Center for Agricultural Utilization Research, ARS- USDA, Peoria, IL, USA.

Bio-oils, biochar preparation

Bio-oils

The bio-oils were obtained from Robert A. Moreau and Charles A. Mullen (Sustainable Biofuels and Co-Products Research Unit, Eastern Regional Research Center, 600 East Mermaid Lane Wyndmoor PA 19038). The 'Carthage' switchgrass cultivar (*Panicum virgatum* L.) was the biomass feedstock for fast-pyrolysis conducted in a bubbling fluidized bed of quartz sand at a temperature of 500°C . The bio-oil collected from the cyclonic condenser and an electrostatic precipitator (ESP) was separated into aqueous and organic phases. A bio-oil mixture phase was prepared by combining the two initial bio-oil phases (condenser aqueous phase and condenser organic phase) on the basis of their respective yields which contained polar and nonpolar compounds dissolving in acetone, while each specific-phase bio-oil contained either polar or nonpolar compounds. All samples were stored in a refrigerator at 4°C until use. The chemical composition of the pyrolysis bio-oil was analyzed by gas chromatography mass spectrometry (GC-MS) (Table 1). Water content was measured by Karl-Fischer titration in methanol with Hydranal Karl-Fischer Composite 5 (Fluka) used as the titrant (Table 1).

Each bio-oil phase (aqueous and organic) and their mixture (aqueous plus organic phase) were extracted in a separatory funnel based on relative solubility using a rotary evaporator (Rotavapor®-R, Brinkmann Butchi,

Switzerland) at 60°C . All phases were separated into six fractions identified by capital letters A- F, so that: A and B fractions were aqueous phases; C and D fractions were organic phase; E and F fractions were mixture phase. The aqueous fractions (A and B) were dissolved in reverse osmosis (RO) water as solvent. The organic fractions (C and D) and mixture fractions (E and F) were dissolved in acetone:water solvent at 2:1 ratio to prepare several desired concentrations. Each fraction was tested for insect mortality.

Biochar

The biochar sample was a product of Biochar Options LCC, Hartland WI, USA, which used hardwood biomass of the red elm, *Ulmus rubra* L. An analysis of hardwood biochar sample was reported by the Midwest Laboratories Inc., Omaha, Nebraska, USA, listing: moisture (2.67%), ammoniacal nitrogen (0.005%), nitrogen total (0.56%), phosphate total (0.14%), loss on organic matter (95.05%), total carbon (88.43%), potash (0.47%), sulfur (<0.05%), calcium (0.36%), magnesium (0.05%), sodium (0.030), iron (70 ppm), manganese (<20 ppm), copper (<20 ppm), zinc (72 ppm), boron (<20 ppm), carbon nitrogen ratio (158:1), ash (2.28%), cation exchange capacity (6.8 meq/100g), conductivity (2.36 mS/cm) and hydrogen (2.34%).

The procedure of preparing biochar sample suspensions was identical to the one described by Wang et al. (2013). Briefly, 15.0 g of biochar was added to 500 ml of DI water, gently stirred for 1 min, then sonicated (100 W, 45 kHz, sonicator, USA) in a water bath for 30 min to disperse the suspension to prepare a biochar stock suspension, and the pH was adjusted to 8.5. The other pHs of biochar suspensions were buffered, using buffers of hydrogen phthalate 0.05 M and potassium dihydrogen phosphate to obtain the final pH 4.0 and 7.1, respectively. The objective was to examine the interactions between different alkaline conditions of spray-dryer feedstock biochar and the tested insect pathogen agents in terms of insect mortality.

Microbial agents

Three different microbial agents used in this work, i.e. the entomopathogenic baculovirus *AgipMNPV*, entomopathogenic bacterium *Bt*, and entomopathogenic fungus *Bb*, were maintained in the National Center for Agricultural Utilization Research (NCAUR), Peoria, IL, USA. Each of the bio-oils and alkaline conditions of biochar combined with the microbial agents was assessed for insecticidal activity.

Table 1. Characteristics of components of the tested bio-oil phases

Compound	ESP (Organic) Conc. (wt%)	Condenser (aq) Conc. (wt%)
Water (KF)	8.05	50.45
Benzene	0.005	0.001
Acetic Acid	4.681	11.580
Acetol	2.053	4.318
Toluene	0.023	0.001
Ethyl Benzene	0.009	0.000
p-xylene	0.009	0.001
2-methyl cyclopentanone	0.004	0.039
o-xylene	0.006	0.000
Furfural	0.051	0.027
fufuryl alcohol	0.003	
2-methyl-2-cylopenten-1-one	0.409	0.141
2(5H)-furanone	0.100	0.079
2,3-dimethyl-2-cyclopenten-1-one	0.029	0.001
3-methyl-1,2-cyclopentandione	0.118	0.001
4-methyl benzaldehyde	0.011	
Phenol	0.681	0.328
Guaiaacol	0.002	0.001
o-cresol	0.147	0.089
Naphthalene	0.097	0.002
p-cresol	0.241	0.071
m-cresol	0.239	0.084
2-methoxy-4-methylphenol	0.001	0.000
2,4-dimethylphenol	0.132	0.030
4-ethyl phenol	0.251	0.053
2-methyl naphthalene	0.075	0.002
5-hydroxymethyl-2-furaldehyde	0.072	
2,6-dimethoxyphenol	0.000	0.000
Vanillin	0.002	
3',5'-dimethoxyacetophenone	0.001	0.001
4-hydroxy-3-methoxyphenylacetone		
Pyridine	0.010	0.006
Styrene	0.044	0.001
Indene	0.084	0.002
Biphenyl	0.014	0.000
Fluorene	0.021	0.000
Anthracene	0.015	0.001
Indane	0.003	0.000
Decalin	0.001	
Tetralin	0.002	0.000
4-hydroxy-2-methylacetophenone	0.011	0.000
Levoglucozan	4.728	0.743
Syringaldehyde	0.001	
Fluoranthene	0.050	0.050

Agrotis ipsilon nucleopolyhedrovirus (AgipMNPV)

The strain *AgipMNPV*, originally isolated from infected late instar larvae of black cutworm, was propagated at NCAUR, Peoria, IL as described by Behle (2015). Frozen infected caterpillars were macerated in 0.1% sodium dodecyl sulfate (SDS) for 10 min, filtered through five layers of cheese cloth, and then centrifuged at 900 x g for 10 min. The pellet contents were re-suspended in 0.5% SDS and centrifuged again. Re-suspension and centrifugation were repeated with 0.5 M NaCl with the final suspension in distilled water. This purified occlusion bodies (OB) suspension was stored at 4 °C. OB concentrations were determined using a phase-contrast microscope and a Neubauer bright-line hemocytometer (Fisher, Pittsburgh, PA).

Bacillus thuringiensis var. kurstaki

The strain of *Bacillus thuringiensis var. kurstaki* used in this study was the currently designated active ingredient of the commercial bioinsecticide registered as Deliver[®] (trademark of Certis USA, L.L.C. 9145 Guilford Road Suite 175 Columbia, MD 21046). This strain was re-isolated from the commercial product and the production system was based on techniques described by Sayed & Behle (2017a,b).

Beauveria bassiana

B. bassiana strain GHA (ARSEF6444) is the active ingredient of the commercial product Mycotrol[®] (Laverlam International Cop., Butte, MT, USA). Liquid fermentation production was used to prepare fresh blastospores for this research, based on techniques reported by Jackson et al. (1997) and Sayed & Behle, (2017a,b).

Experimental procedures

Three experiments were conducted, two experiments with bio-oils and one with biochar, to assess different bio-oil fractions (aqueous, organic and mixture) against the 1st larval instar of black cutworm, *A. ipsilon* (experiment I). Based on the results of experiment I for bio-oil phases, experiment II examined the effects of bio-oil fractions, each at 2% and 4% concentration, mixed with median lethal concentrations LC₅₀ of the three different microbial agents: 1.2 × 10⁵ OB ml⁻¹ for baculovirus *AgipMNPV*, 3.85 × 10⁷ spores ml⁻¹ for *Bt*, and 3.58 × 10⁷ spores ml⁻¹ for *Bb* as determined by Behle (2015) and Sayed & Behle (2017a). Experiment

III was performed to compare the pHs of spray-dried formulation ingredients including biochar mixed with the microbial agents regarding their insecticidal activity.

Insecticidal activity of bio-oils phases

Dosage-response bioassays of the bio-oils, each as an aqueous, organic or mixture fraction, were performed using the leaf disk assay to estimate the median lethal concentrations for each bio-oil fraction under laboratory conditions. Insect mortality was assessed for neonates of black cutworm, *A. ipsilon*, when exposed to treated cabbage leaf disks. Aqueous suspensions of each bio-oil fraction were prepared and desired concentrations were adjusted through serial dilutions using reverse osmosis (RO) water for the aqueous fraction, and RO water plus acetone at 1:2 ratio for each of the organic and mixture fractions, to make serial dilutions delivering seven concentrations (wt/wt) of 50%, 25%, 12.5%, 6.25%, 3.12, 1.78 and 0.78%, for each bio-oil fraction.

Leaf disks (3.8 cm diameter) were excised from 4–6 week old greenhouse-grown cabbage, *Brassica oleracea*, cv. 'Bravo F1 Hybrid' (Harris Seeds, Rochester, NY, USA). Disks were cut from cabbage leaves with a cork borer and placed individually (top-side up) on filter paper disks (Whatman No. 1, Maidstone, England) placed in 50 × 9 mm Petri dishes with tight fitting lids (Falcon, Becton Dickson and Company, Franklin Lakes, NJ). Five leaf disks were prepared and considered as five replications for determining mortality for each treatment concentration. The leaf disks with controls consisted of RO water, acetone, and RO water plus acetone at 1:2 ratio, and the solutions were included to assess insect mortality due to handling and to examine the impact of solvents. Each leaf disk received 100 µL of each bio-oil fraction which was spread evenly over its surface with a glass rod and allowed to air dry. Control samples were treated with solvents alone. Ten 1st instar larvae of *A. ipsilon* were placed on each treated leaf disk with a total 50 larvae for each treatment dosage.

All insect bioassays were held in the dark at 28 °C, 70% relative humidity and a photoperiod of 14L:10D for five days before assessing mortality. Mortality of larvae was recorded daily after 1, 2, 3, 4 and 5 days. The entire experiment was repeated three times on different dates using different insect cohorts. Larvae were considered dead if they did not respond to contact. Dosage response mortality data were analyzed using Polo Plus software (LeOra, Palo Alto, CA, USA) to determine the median lethal concentration (LC₅₀) values after 3 days. Damage to the treated leaves caused by the bio-oils was visually assessed.

Insecticidal activity of bio-oils phases mixed with entomopathogenic baculovirus (AgipMNPV), bacterium (Bt) and fungus (Bb)

The aqueous, organic and mixed bio-oil phases were serially diluted to 4% and 2% concentrations for insecticidal assays. Both concentrations were used based on the approximate median and high lethal mortality results of the six bio-oil fractions. Each dilution of bio-oil fraction was gently stirred to mix it with the median lethal concentration (LC₅₀) of each microbial agent based on previous results obtained by Sayed & Behle (2017a) and Behle (2015).

Droplet-feeding bioassays to determine the insecticidal activity of various treatments of bio-oil fractions, each at 4% and 2% concentration, mixed with the LC₅₀ of black cutworm virus (*AgipMNPV*) at 1.2×10^5 OB ml⁻¹ were performed using procedures reported by Behle (2015). In general, each virus-supplemented bio-oil sample was mixed into 10 ml of a feeding solution containing 2% (w/w) sucrose and 0.1% dye (w/w) FD&C Blue 1 (Noveon Hilton Davis, Cincinnati, OH). After mixing, about 60 small drops (≈ 0.5 ml) from each sample were placed in individual plastic 9x50-mm Petri dishes, five Petri dishes making each treatment. Then about 15 neonates of *A. ipsilon* were placed in each dish to feed on the droplets, and the Petri dishes were capped to reduce evaporation. After feeding for 5 min, six larvae with blue-stained intestines from each dish were transferred to individual cups containing 3 ml artificial diet to fill a tray of 30 cups, and were incubated for 7 days at 28°C and 55% RH in a dark Conviron I24 L incubator (Controlled Environments, Inc., Asheville, NC). After incubation, live and virus-killed larvae were counted to calculate the mortality percentage for each treatment. Dead larvae that were not symptomatic for virus infection were omitted from statistical analysis.

Modified insect exposure techniques were used to preferentially evaluate mortality caused by the bio-oil fractions (at 4% and 2% concentrations) mixed with the LC₅₀ each of the *Bt* at 3.85×10^7 spores ml⁻¹, and *Bb* at 3.58×10^7 blastospores ml⁻¹ (determined previously, unpublished data, AMMS). Cabbage leaf disks were prepared as described for experiment I in *Experimental procedures*. Each leaf disk received 100 μ l of sample that was spread evenly over the surface with a glass rod and allowed to air dry. Once the treatments dried, each Petri dish was infested with 10 neonate larvae of *A. ipsilon* incubated (Conviron I24 L, Controlled Environments Incorporated, Pemia, NC, USA) in the dark at 28°C for exposure to treated leaf disks.

To effect mortality by *Bt* with bio-oil fractions, neonates were incubated on treated leaf disks for 72 h to provide continuous exposure to treated leaf tissue before assessing mortality. At least five Petri dishes with 10 larvae per dish (50 total larvae) were evaluated for each treatment, with each dish considered as a replication for determining mortality. Continuous exposure to treated leaf tissue maximized larval consumption, and the susceptible larvae died during the short 3-day incubation. However, this 3-day exposure technique was not suitable for evaluating slower acting fungal infections.

To effect mortality by *Bb* with bio-oil fractions, larvae were incubated on treated leaf disks for a limited 24 h exposure period, then six larvae from each of the five dishes were transferred to individual diet cups to fill a tray of 30 cups for each sample treatment. The diet cups contained 3 ml artificial diet and allowed larvae to feed for 4 additional days without additional exposure to the treatments before assessing mortality. A tray of 30 larvae was considered as the replicate for determining mortality for each treatment, and a minimum of three replicates were used to assess mortality in each treatment (n=90 larvae per treatment). For both exposure techniques, larvae were considered dead if they did not respond to contact.

Controls consisting of the aqueous, organic and mixture bio-oil phases at both dilution concentrations (4 and 2%), and Ro water and Ro water plus acetone (1:2) solutions, served to assess insect mortality.

Impact of pHs on insecticidal activity of spray-dried formulations containing biochar with entomopathogenic viruses, bacteria and fungi

Interactions among the acidic/alkaline conditions of spray-dried feedstocks containing biochar made with three different entomopathogens: baculovirus *AgipMNPV*, bacterium *Bt* and fungus *Bb*, were examined for insecticidal activities.

Three aliquots of biochar solutions with each of the used entomopathogens were adjusted to provide samples at pH 4.0, 7.1 and 9.6 by adding standard buffers. Biochar was dissolved in water with added standard buffer pH=4.0 potassium phthalate 0.05M, potassium dihydrogen phosphate pH=7.0, and without a buffer to produce final pHs of 4.0, 7.1 and 9.6, respectively. Nine biochar solutions mixed with each microbial formulation of baculovirus black cutworm, *AgipMNPV* at 2.2×10^9 occlusion bodies (OB) ml⁻¹, *Bt* at 2.63×10^9 spores ml⁻¹, and *Bb* at 3.3×10^9 blastospores ml⁻¹, were used to make spray-dried samples (Table 2).

Table 2. Ingredients and product yields of spray-dried formulations of biochar made with entomopathogenic baculovirus *AgipMNPV*, bacterium *Bacillus thuringiensis* and fungus *Beauveria bassiana*

Microbial agent	Ingredient	Biochar	Biochar	Bochar
	Biochar	2.5 g		
	Biochar		2.5 g	
	Biochar			2.5 g
	Buffer pH = 4.0 (Potassium Hydrogen Phthalate 0.05 M)	20 ml		
	Buffer pH = 7.0 (Potassium dihydrogen phosphate)		100 ml	
	pH	4.0	7.1	9.6
	Expected total weight	2.7 g	3.5 g	2.5 g
	Final volume at 2.5% solids	100 ml	100 ml	100 ml
<i>AgipMNPV</i>	Unformulated virus stock 2.2e9 OB ml ⁻¹	3.4 ml	2.4 ml	1.75 ml
	Water activity (a _w) at temperature degree	0.241	0.297	0.356
	Yield %	41.11	20.29	100
<i>Bacillus thuringiensis</i> var. <i>kurstaki</i>	Unformulated bacterial stock 5.3 e9 spores and crystals g ⁻¹	1.2 ml	1.4 ml	1.15 ml
	Water activity (a _w) at temperature degree	0.241	0.297	0.356
	Yield %	58.0	48.0	71.0
<i>Beauveria bassiana</i>	Unformulated blastospores stock 2.2e9 blastospores ml ⁻¹	1.72 ml	2.8 ml	3.5 ml
	Water activity (a _w) at temperature degree	0.241	0.297	0.356
	Yield %	55.0	50.0	39.0

All spray-dried formulations of feedstock suspensions (100 ml) were dried in a Niro atomizer portable spray dryer (GEA-Niro, Copenhagen, Denmark). Standard spray-drying conditions were: 20 ml min⁻¹ feed rate, 5.8 bar (8 kg/cm²) air pressure at the inlet-outlet temperatures of 130-135°C to 60-70°C for the baculovirus *AgipMNPV*, 90-95°C to 50-60°C for the bacterium *Bt*, and 95-100°C to 50-60°C for the fungus *Bb*. The suspensions were stirred during the spray-drying process to prevent settling. The percentage of all powders recovery was calculated by taking the difference between the final weight of the powder collected and the weight of the solids in the initial suspension. Water activity (a_w) of each spray-dried formulation sample was determined with a water activity meter (AquaLab series 4TEV). Insecticidal activity of these formulation samples was compared using a single-dosage assay representing the LC₅₀ for each unformulated microorganism, i.e. virus, bacterium and fungus.

Droplet-feeding application to determine the insecticidal activity of various treatments with the baculovirus was performed as previously described. The bioassay of each spray-dried formulation using droplet method was performed by using a blue solution containing 2% sugar and 0.1% blue dye. The resulting

suspensions should all have contained 1.2 x 10⁵ OB ml⁻¹, and represented the LC₅₀ of the unformulated virus. Three trays of 30 larvae (90 larvae in total) were considered as three replicates for determining mortality for each treatment. Larvae in cups were stored at 28°C in the dark incubator for assessing mortality after 7 days exposure to viral treatments. Larvae were considered dead if they did not respond to contact. Dead larvae that were not symptomatic for virus infection were omitted from statistical analysis. A no-virus control was included with each assay to indicate handling mortality of the larvae.

Suspensions of the spray-dried bacterial formulations made with biochar were prepared by adding 0.007 g of each powder formulation into 10 ml of 0.02% aqueous Silwet surfactant solution to provide the LC₅₀ concentration of 3.85 × 10⁷ spores ml⁻¹ for application to leaf disks. Five cabbage leaf disks per treatment were prepared, infested with 10 larvae each, and the larvae were allowed to feed for 72 h. Then Petri dishes with 10 larvae each per treatment (50 total larvae) were evaluated and considered as five replicates for determining the mortality for each treatment. The procedures were repeated three times in different periods and the number of live and dead larvae were recorded.

Insecticidal activity of the fungal formulation samples were compared using a single-dosage assay representing the LC₅₀ of the unformulated fungus. Each spray-dried formulation was diluted at a rate of 0.02 g in 10 ml of deionized water to provide a concentration of 3.58 × 10⁷ blastospores ml⁻¹. After one day of exposure to treated leaf disks, six larvae per dish were transferred to individual diet cups and incubated in the dark at 28° C. Live and dead larvae were counted daily up to five days after exposure. The experiment was repeated three times at different dates to provide three trays of 30 larvae (90 larvae in total) each per treatment that were considered as three replicates.

Statistical analysis

Dose response data from bioassays were subjected to probit analysis using Polo Plus, Version 2.0 (LeOra Software, Petaluma, CA) to calculate the median lethal concentrations of LC₅₀, with corresponding confidence limits (95% CL) and slope for each bio-oil fraction. Mortality due to bio-oil fractions, individually and mixed with three microbial agents, was based on five replicates of Petri dishes with 10 larvae due to *Bt*, and three replications, 30 larvae per replication, due to each of *Agip*MNPV and *Bb*. The average of repeated three times was taken for statistical analysis. Dead larvae that were not symptomatic for any of the three infections were omitted from statistical analysis. No-treatment controls were included with each assay to indicate handling mortality, which typically averaged less than 2% and thus data were not corrected for control mortality. Paired *t*-test was used to analyze the differences in the activity of bio-oil fractions only and with the three incorporated microbial agents.

The effect of alkaline conditions of spray-dried biochar combined with the tested microbial agents on larval mortality was subjected to analysis of variance and treatment means were separated using the least significant difference (LSD) test at 0.05, and processed with the statistical software SPC for Excel (Knoware International, Inc., Denver, CO, USA).

RESULTS

Insecticidal activity of bio-oil fractions

All bio-oil fractions other than the aqueous fraction B, organic fraction D and mixture fraction E were found to have impact against *A. ipsilon*. The highest mortality was achieved by the organic fraction C, followed by mixture fraction F and aqueous fraction A. The dosage response bioassay for the six fractions of bio-oils provided data suitable for probit analysis. The median lethal concentration ratios of LC₅₀ compared to fraction A (2.77 mg ml⁻¹) were 0.23 for aqueous fraction B, 2.41 for organic fraction C, 0.21 for organic fraction D, 0.06 for mixture fraction E and 0.90 mg ml⁻¹ for mixture fraction F as shown in Table 3 and Figures 1 and 2.

Among the six fractions tested as illustrated in Figure 1, the organic fraction C revealed the greatest activity over all other fractions. Organic fraction C caused 100% larval mortality of black cutworm at the serial dilution concentrations of 50, 25, 12.5 and 6.25% within 1 day, and 3.125% at the 2-day evaluation. Fraction C diluted to 1.52 and 0.78% concentrations reduced the mortality rates considerably, i.e. to 42 and 26%, respectively, within 3 days. The same trend of results

Table 3. Probit analysis for insecticidal activity against 1st larval instar of *Agrotis ipsilon* by six bio-oil fractions

Bio-oil fraction ^a	Lethal concentration ^a (Lower-upper of fiducial limits) mg ml ⁻¹	Slope ^b ± standard error (SE)	Chi-square goodness of fit (X ²)	Heterogeneity
Aqueous Fraction A	2.77 (1.66–4.50)	2.69 ± 0.28	5.88	2.44
Aqueous Fraction B	0.23 (0.18–0.29)	2.35 ± 0.21	7.30	1.83
Organic Fraction C	2.41 (1.94–2.98)	4.51 ± 0.64	3.48	0.70
Organic Fraction D	0.21 (0.17–0.26)	3.98 ± 0.42	7.21	1.84
Mixture Fraction E	0.06 (0.04–0.11)	4.03 ± 1.09	6.65	3.23
Mixture Fraction F	0.89 (0.70–1.13)	2.73 ± 0.30	1.59	0.79

^a Total number of neonates of black cutworm, *A. ipsilon* tested 150 larvae, five replicates (10 larvae per replicate)/repetition/concentration
^a Delivered median lethal concentration (LC₅₀) expressed by infective mg ml⁻¹ and estimated by the logistic model, lethal dose ratios (LC₅₀) compared to Fraction A. Cumulative mortality censored up to day 5. Mortality was none in controls.

^b Slope for mortality represents regression of proportion of larval mortality versus log₁₀ of mg ml⁻¹

was demonstrated by the other fractions after 2, 3, 4 and 5 days of testing. It is worth noting that mixture fraction E did not cause mortality after 1, 2 and 3 days as shown in Figure 1.

Effects of bio-oil fractions mixed with microbial agents

The activity of the bio-oil fractions at dilution concentrations of 4% and 2% was determined

independently and in combination with the LC₅₀ of three microbial agents: bacterium *Bt*, fungus *Bb* and baculovirus *AgipMNPV*.

Considering the insecticidal activities of treatments with the organic and mixture bio-oil fractions at 4% concentration, the greatest mortality of 100% was observed after three days of assay. However, at the same concentration, mortality was observed to decline to 62.08, 48.67, 83.33% and 94.87, 69.44, 71.00% when the same organic and mixture bio-oil fractions

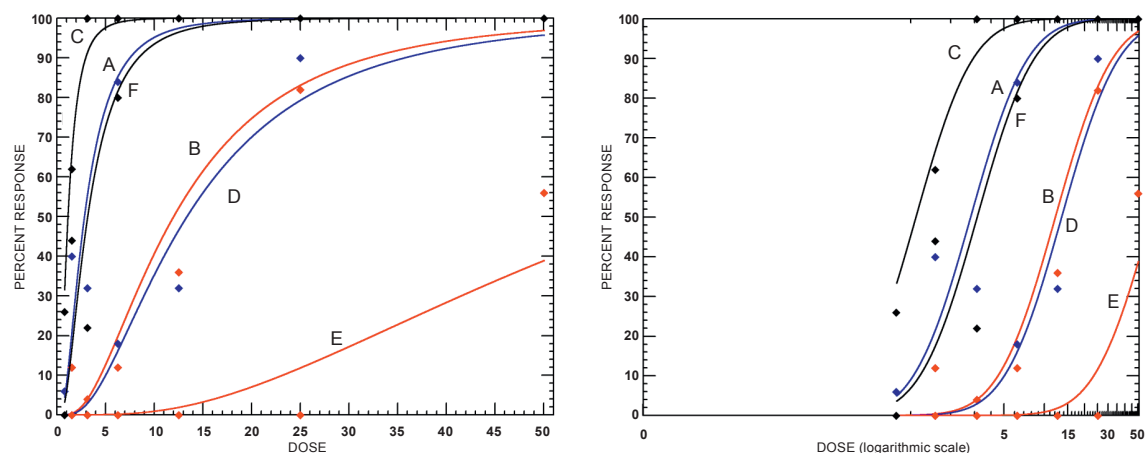


Figure 1. Dose-response curves of six bio-oil fractions against 1st larval instar of *Agrotis ipsilon*. Data are estimated by lethal dose ratios and logistic model, where A and B fractions were aqueous phases; C and D fractions were organic phase, E and F fractions were the mixture phase

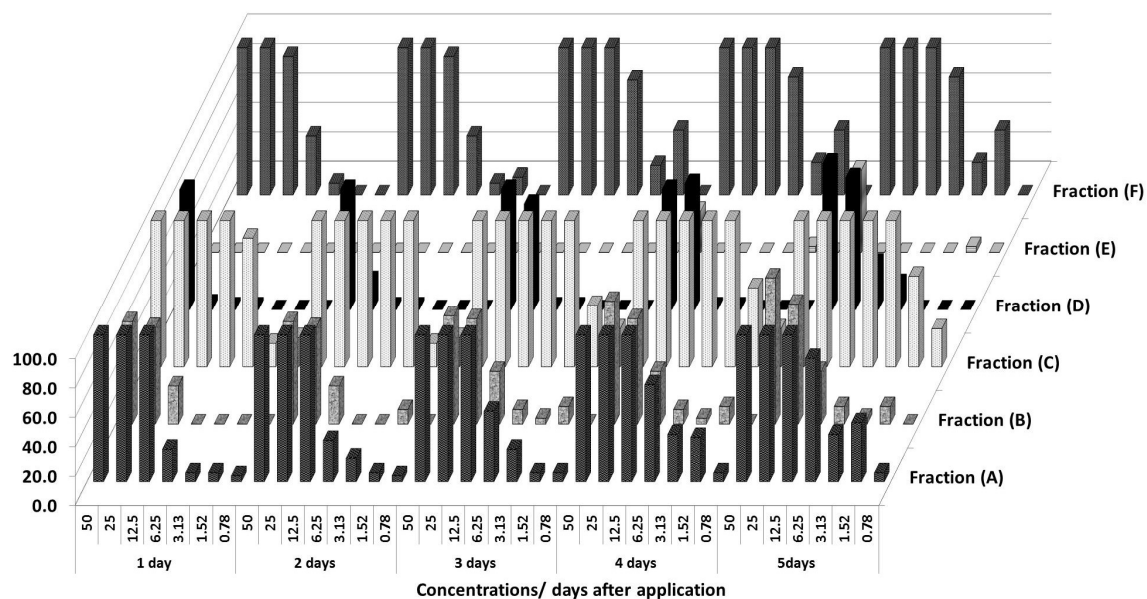


Figure 2. Comparison of mortality percentages for *Agrotis ipsilon* larvae exposed to six bio-oil fractions: aqueous A, aqueous B, organic C, organic D, mixture fraction E and mixture fraction F

were combined with the three entomopathogenic agents, i.e. baculovirus *AgipMNPV*, bacterium (*Bt*) and fungus (*Bb*), respectively, as shown in Table 4. It is obvious that the mixtures had significantly different activity based on the microbial agents *Bt* ($F_{5,29}=4.19$, $P=0.007$), *Bb* ($F_{5,17}=4.12$, $P=0.023$) and baculovirus *AgipMNPV* ($F_{5,17}=5.45$, $P=0.008$). However, highly significant effects were found when the bio-oil fractions were used independently ($F_{5,17}=11.16$, $P=0.0004$), i.e. without mixing with the microbial agents (Table 4).

By contrast, the comparison of bio-oil fractions only and in combination with the three microbial agents indicated no significant differences in insecticidal efficacy for baculovirus *AgipMNPV* seven days ($t=0.410$, $df=10$, $P=0.690$), *Bt* three days ($t=0.992$, $df=9$, $P=0.334$), and *Bb* five days ($t=1.784$, $df=10$, $P=0.105$) after initial exposure. Overall, the bio-oil fractions applied alone tended to cause greater mortality of the exposed larvae than did any mix with the microbial agents. The bio-oils were not observed to cause damage to the treated leaves.

Effects of pH of biochar mixed with microbial formulations

The spray-dried formulation ingredient of biochar mixed with three microbial agents (*AgipMNPV*, *Bt* and *Bb*) had various effects based on the alkalinity of dryer feedstock and the microbial agents. There were significantly different levels of activity based on mixtures with the baculovirus *AgipMNPV* ($F_{2,9}=35.02$, $P<0.0005$), *Bt* ($F_{2,14}=5.20$, $P=0.024$), and *Bb* ($F_{2,8}=8.81$, $P=0.016$). However, no significant difference was observed when only biochar ($F_{2,8}=6.30$, $P=0.054$) was used at three pH levels without adding the microbial agents (Table 5).

It is obvious that the spray-dried formulation ingredient of biochar formulation mixed with baculovirus *AgipMNPV*, *Bt* and *Bb* at pH 7.1 had significantly higher insecticidal effects of 64.93, 65.84 and 62.87 %, respectively, compared with the biochar formulation made in combination with the same agents at pH 4.0 (36.04, 39.53 and 42.22 %, respectively), and pH 9.6 (3.76, 21.17 and 54.56 %, respectively), whereas biochar without the microbial agents had the lowest effect of 4.37% at pH 7.1.

Table 4. Insecticidal activity of aqueous, organic and mixture bio-oil phases at two concentrations, 4% and 2%, against *Agrotis ipsilon* larvae, exposed only to bio-oils and to their combination with the median lethal concentration (LC₅₀) of microbial agents *AgipMNPV*, *Bacillus thuringiensis* var. *kurstaki* and *Beauveria bassiana*

Bio-oil phase	Concentration	Bio-oil phase*			
		Individually	Mixed with microbial Agents		
			<i>AgipMNPV</i>	<i>Bacillus thuringiensis</i>	<i>Beauveria bassiana</i>
Aqueous	4%	50.56 ± 2.42 bc	64.60 ± 3.09 bc	48.20 ± 3.50 b	62.45 ± 4.59 ab
	2%	42.22 ± 2.01 c	46.88 ± 4.50 c	52.05 ± 2.33 ab	36.71 ± 4.79 b
Organic	4%	100.00 a	62.08 ± 2.37 bc	48.67 ± 3.76 b	83.33 ± 1.66 a
	2%	56.67 ± 4.16 bc	55.01 ± 1.51 c	41.00 ± 1.87 b	43.00 ± 3.87 b
Mixture	4%	100.00 a	94.87 ± 5.13 a	69.44 ± 4.65 a	71.00 ± 4.38 ab
	2%	83.33 ± 4.41 ab	78.33 ± 3.16 ab	54.03 ± 0.39 ab	61.58 ± 3.09 ab
Control	Ro	00.00	00.00	00.00	00.00
	Ro + acetone	2.59 ± 0.74	1.16 ± 0.41	00.00	77.77 ± 0.22

* Means marked with the same letter in each column are not significantly different (LSD). 62.08, 48.67, 83.33% and 94.87, 69.44, 71.00

Table 5. Comparison of mortality percentages (main effect±se) for *Agrotis ipsilon* larvae exposed to spray-dried formulation of biochar at pH 4.0, 7.1 and 9.6, and mixed with the median lethal concentrations (LC₅₀) of microbial agents *AgipMNPV*, *Bacillus thuringiensis* and *Beauveria bassiana*

Ingredient Formulation*	pH	Mixed with LC ₅₀ of microbial agents*			Without adding microbial agents
		<i>AgipMNPV</i>	<i>Bacillus thuringiensis</i>	<i>Beauveria bassiana</i>	
Biochar	4.0	3.77 ± 1.54 b	27.82 ± 2.25 b	57.11 ± 3.33 ab	2.20 ± 0.89 ab
	7.1	65.41 ± 3.28 a	64.54 ± 2.32 a	63.52 ± 1.11 a	4.37 ± 1.15 a
	9.6	20.80 ± 0.32 b	26.41 ± 0.41 b	43.39 ± 0.68 c	0.56 ± 0.13 b
Controls		00.00	0.98 ± 0.02	00.00	00.00

* Means marked with the same letter in each column are not significantly different (LSD).

DISCUSSION

The results of this research support earlier findings about good potentials for using pyrolysis products as source material for making insecticides, and for finding alternative compounds to synthetic chemical pesticides.

In the bioassay tests, the organic bio-oil phase was the most active liquid causing 100% mortality of cutworm larvae after 24 h and it killed larvae faster than either the aqueous or mixture phase. It indicates that the most active components are imbedded in the organic fraction. Pyrolysis oils contain many organic components, such as: 1) cellulose/hemicellulose derived compounds (acetic acid, furfural, hydroxyacetaldehyde, acetol, levoglucosan); 2) lignin derived compounds (guaiacol, isoeugenol, 2,6-dimethoxyphenol, phenol); 3) protein derived compounds (pyrrole, benzylnitrile, indole). It is impossible to say which are the most effective ones because of the complexity of synergistic interactions among them. However, our results support some earlier studies (Hossain et al., 2013) indicating that lignin-based bio-oils have higher insecticidal activity than those produced from hemicelluloses and cellulose.

An interesting observation was that the black cutworm larvae ate little or none of the treated leaves. In addition, the application seemed to be slightly phytotoxic. These results strongly suggested that a variety of chemicals in bio-oil fractions are active (repell, kill) in controlling the black cutworm *A. ipsilon*. The bio-oil organic phase had much higher levels of levoglucosan, acetic acid, acetol, phenol, 2-methyl-2-cyclopenten-1-one, 4-ethyl phenol, p-cresol, m-cresol, o-cresol, 2,4-dimethylphenol, 3-methyl-1,2-cyclopentandione, and 2(5H)-furanone, as well as less aromatic compounds, and less nitrogen-containing compounds than the other phases.

The great increase in mortality observed for each fraction indicated that the active compounds in these fractions required a short ingestion period to reach a toxic dose for *A. ipsilon* larvae. It was also notable that the application of these fractions greatly reduced the appetite of larvae which could have an effect on larval mortality. The compounds that caused mortality were the same as some known to act as anti-feedants or repellants (Hagner et al., 2015). Other studies have demonstrated that wood vinegar repels other species, e.g. psyllids (*Trioza apicalis*) and termites (*Reticulitermes speratus* and *Coptotermes formosanus*) (Oramahi & Yoshimura, 2013).

Booker et al., (2010b) demonstrated that the Colorado potato beetle, *Leptinotarsa decemlineata* L. (Coleoptera: Chrysomelidae), was strongly affected by tobacco bio-oil (organic and aqueous phases). The adjusted *L. decemlineata* 2nd larval instar mortality 48 h after treatment was always

higher for the organic than for the aqueous phase bio-oil. Suqi et al. (2014) evaluated the insecticidal and feeding repellent activities of bio-oil pyrolysed from mustard straw for control of Colorado potato beetle larvae. Hossain et al. (2015) revealed that lignin bio-oil provided a broad spectrum activity and was effective against two insects (*L. decemlineata* and *Trichoplusia ni*), three fungal species (*Pythium ultimum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*), and three bacterial species (*Clavibacter michiganensis* subsp. *michiganensis*, *Streptomyces scabies* and *Xanthomonas campestris* var. *vesicatoria*).

Chemical composition of bio-oils depends on many factors, such as biomass type, feedstock pre-treatment (particle size and shape, moisture and ash contents), pyrolysis conditions (temperature, heating rate, residence time, pressure, gaseous environment), as well as vapor filtration and condensation (filter type, condensing method and medium, cooling rate). Therefore, bio-oils produced from different materials and by different pyrolysis reactors may differ greatly one from another (Lu et al., 2009).

Bio-oil is corrosive as it contains mainly acids, alcohols, ketones, aldehydes, phenols, ethers, esters, sugars, furans, nitrogen compounds and multifunctional compounds. Separation of bio-oil components responsible for pesticide activity has identified many small molecules, such as phenol derivatives (Booker et al., 2010a; Bedmutha et al., 2011) and fatty acids (Suqi et al., 2014). Phenols, alcohols, aldehydes and formic acids were identified in bio-oils from pyrolysed wood lignin (Liu et al., 2008), while pyrolysis of hemicellulose releases a mixture of acids, aldehydes, alkanes and ethers (Yang et al. 2007). Bio-oils produced from pyrolysis of greenhouse tomato plant residues have been suggested to be good starting material for insecticide products (Cáceres et al., 2015).

Another important finding of this study was that all three bio-oil phases were more active when combined with the microbial agents. This may indicate that pyrolysis oils have an antagonistic impact on the microbial control organisms. Antifungal efficiency was reported to be strongly dependent on phenolic compounds (Jung, 2007; Baimark & Niamsa, 2009).

The results support the idea that antagonistic is a function of the proportions of components in the mixture, where one proportion may be synergistic, while another is simply additive. In previous studies, Jung (2007), Bedmutha et al. (2011) and Booker et al., (2010b) indicated that bio-oil compounds may play a role in anti-microbial activity along with the main biomass components.

The third round of experiments clearly showed higher mortalities from the spray-dried biochar formulation mixed with microbial agents at pH 7.1 than those obtained

at pH 4.0 and 9.6. The highest mean mortality was obtained at pH 7.1, indicating a great insecticidal activity under neutral conditions. No similar results have been published before. It is inferred that biochar may play a vital role in supporting alternatives and could be an appropriate additive or even useful substitute for pesticides, and it has high structural stability and likely some other favorable properties, such as air capacity and water-holding capacity.

Due to many negative side-effects of synthetic pesticides, there is an urgent need for novel active compounds as safe alternatives to harmful pesticides. Novel biopesticides should overcome pesticide resistance and be compatible with integrated pest management practices. In addition, such compounds should possess high selectivity, so that their use can be safe for humans and the environment (Hagner et al., 2013).

Furthermore, there is an increasing interest in developing pyrolysis technology as a “greener” solution to produce energy and chemicals using local natural biomass as feedstock. It is obvious that pyrolysis liquids can be used as raw material for making repellents, insecticides, molluscicides, herbicides and fungicides. In most of such products, their efficacy is based on a mixture of many components. However, wide use of pyrolysis liquids as biopesticides will require many changes in pesticide registration procedures (Tiilikka et al., 2011). Further research is required to identify the active compounds able to contribute to insecticidal activity, potentially produced by pyrolysis of different types of biomass at low production cost. Environmental safety and impact on beneficial organisms must always be considered prior to their recommendation for use in agricultural and IPM programs.

ACKNOWLEDGEMENTS

This cooperative research was made possible by the Agricultural Research Center (ARC), Plant Protection Research Institute (PPRI), Egypt and the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), National Center for Agricultural Utilization Research (NCAUR), USA. We extend thanks to Robert A. Moreau, and Charles A. Mullen, USDA, Sustainable Biofuels and Co-Products Research Unit, Eastern Regional Research Center, 600 East Mermaid Lane Wyndmoor PA 19038, USA, for their assistance.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

DISCLAIMER

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. The USDA is an equal opportunity provider and employer.

REFERENCES

- Baimark, Y. & Niamsa, N. (2009). Study on wood vinegars for use as coagulating and antifungal agents on the production of natural rubber sheets. *Biomass and Bioenergy*, 33(6-7), 994-8.
- Bedmutha, R., Booker, C.J., Ferrante, L., Briens, C., Berruti, F., Yeung, K.K.C. ... Conn, K. (2011). Insecticidal and bactericidal characteristics of the bio-oil from the fast pyrolysis of coffee grounds. *Journal of Analytical and Applied Pyrolysis*, 90(2), 224-231.
- Behle, R.W. (2015). Insecticidal parameters for the baculovirus infecting *Agrotis ipsilon* neonates. In *International Congress on Invertebrate Pathology and Microbial Control and the 48th Annual Meeting of the Society for Invertebrate Pathology*. Vancouver, Canada.
- Booker, C.J., Bedmutha, R., Scott, I.M., Conn, K., Berruti, F., Briens, C., & Yeung, K.K.C. (2010a). Bioenergy II. Characterization of the pesticide properties of tobacco bio-oil. *International Journal of Chemical Reaction Engineering*, 8. doi: <https://dx.doi.org/10.2202/1542-6580.1939>.
- Booker, C.J., Bedmutha, R., Vogel, T., Gloor, A., Xu, R., Ferrante, L. ... Briens, C. (2010b). Experimental investigations into the insecticidal, fungicidal, and bactericidal properties of pyrolysis bio-oil from tobacco leaves using a fluidized bed pilot plant. *Industrial and Engineering Chemistry Research*, 49(20), 10074-10079.
- Caballero, P., Alamo, S., Vargas-Osuna, E., Santiago-Alvarez, C., & Lipa, J.J. (1993). Biology of *Cotesia* (= *Apanteles*) *telongai* (Hymenoptera: Braconidae) on its natural host *Agrotis segetum* (Lepidoptera: Noctuidae). *Biocontrol Science and Technology*, 3(4), 481-489.
- Cáceres, L.A., McGarvey, B.D., Briens, C., Berruti, F., Yeung, K.K.C., & Scott, I.M. (2015). Insecticidal properties of pyrolysis bio-oil from greenhouse tomato residue biomass. *Journal of Analytical and Applied Pyrolysis*, 112, 333-340.
- Campisi, T., Samorì, C., Torri, C., Barbera, G., Foschini, A., Kiwan, A. ... Pasteris, A. (2016). Chemical and ecotoxicological properties of three bio-oils from pyrolysis of biomasses. *Ecotoxicology and Environmental Safety*, 132, 87-93.
- Chandler, D., Bailey, A.S., Tatchell, G.M., Davidson, G., Greaves, J., & Grant, W.P. (2011). The development,

- regulation and use of biopesticides for integrated pest management. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 366(1573), 1987-1998.
- Czernik, S., & Bridgwater, A.V. (2004). Overview of applications of biomass fast pyrolysis oil. *Energy and Fuels*, 18(2), 590-598.
- El-Salamouny, S., Lange, M., Jutzi, M., Huber, J., & Jehle, J.A. (2003). Comparative study on susceptibility of cutworms (Lepidoptera :Noctuidae) to *Agrotis segetum* nucleopolyhedrovirus and *Agrotis ipsilon* nucleopolyhedrovirus. *Journal of Invertebrate Pathology*, 84(2), 75-82.
- Fagnäs, L., Kuoppala, E., Tiilikkala, K., & Oasmaa, A. (2012). Chemical composition of birch wood slow pyrolysis products. *Energy and Fuels*, 26 (2), 1275-1283.
- Gokce, C., Yilmaz, H., Erbas, Z., Demirbag, Z., & Demir, I. (2013). First record of *Steinernema kraussei* (Rhabditida: Steinernematidae) from Turkey and its virulence against *Agrotis segetum* (Lepidoptera: Noctuidae). *Journal of Nematology*, 45(4), 253-259.
- Gupta, S., & A.K. Dikshit (2010). Biopesticides: An ecofriendly approach for pest control. *Journal of Biopesticides*, 3(1 si), 186-188.
- Hagner, M. (2013). Potential of the slow pyrolysis products birch tar oil, wood vinegar and biochar in sustainable plant protection -pesticidal effects, soil improvement and environmental risks. Doctoral dissertation, pp 42. Department of Environmental Sciences, Lahti, University of Helsinki, Finland.
- Hagner, M., Kuoppala, E., Fagnäs, L., Tiilikkala, K., & Setälä, H. (2015). Using the copse snail *Arianta arbustorum* (Linnaeus) to detect repellent compounds and the quality of wood vinegar. *International Journal of Environmental Research*, 9(1), 53-60.
- Hong S.C., & Williamson, R.C. (2004). Comparison of sticky wing and cone hemomene traps for monitoring seasonal abundance of black cutworm adults and larvae on golf courses. *Journal of Economic Entomology*, 97(5), 1666-1670.
- Hossain, M.M., Scott, I.M., McGarvey, B.D., Conn, K., Ferrante, L., Berruti, F. & Briens, C. (2013). Toxicity of lignin, cellulose and hemicellulose-pyrolyzed bio-oil combinations: Estimating pesticide resources. *Journal of Analytical and Applied Pyrolysis*, 99, 211-216.
- Hossain, M.M., Scott, I.M., McGarvey, B.D., Conn, K., Ferrante, L., Berruti, F. & Briens, C. (2015). Insecticidal and anti-microbial activity of bio-oil derived from fast pyrolysis of lignin, cellulose, and hemicelluloses. *Journal of Pest Science*, 88(1), 171-179.
- Hou, X., Meng, L., Li, L., Pan, G., & Li, B. (2015). Biochar amendment to soils impairs developmental and reproductive performances of a major rice pest *Nilaparvata lugens* (Homoptera: Delphacidae). *Journal of Applied Entomology*, 139(10), 727-733.
- Jackson, M.A. (1997). Optimizing nutritional conditions for the liquid culture production of effective fungal biological control agents. *Journal of Industrial Microbiology Biochemistry*. 19(3), 180-187.
- Jung, K.H. (2007). Growth inhibition effect of pyrolygneous acid on pathogenic fungus, *Alternaria mali*, the agent of *Alternaria* blotch of apple. *Biotechnology and Bioprocess Engineering*, 12(3), 318-322.
- Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M., & Goettel, M.S. (2015). Insect pathogens as biological control agents: Back to the future. *Journal Invertebrate Pathology*, 132, 1-41.
- Lacey, L.A., & Kaya, H.K. (eds.) (2007). Field manual of techniques in invertebrate pathology: Application and evaluation of pathogens for control of insects and other invertebrate pests (2nd edition). Dordrecht, The Netherlands: Springer.
- Laird, D.A., Brown, R.C., Amonette, J.E., & Lehmann, J. (2009). Review of the pyrolysis platform for coproducing bio-oil and biochar. *Biofuels, Bioproducts and Biorefining*, 3, 547-562.
- Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., & Crowley, D. (2011). Biochar effects on soil biota: A review. *Soil Biology and Biochemistry*, 43(9), 1812-1836.
- Liu, Q.S., Wang, S., Zheng, W.Y., Luo, Z., & Cen, K. (2008). Mechanism study of wood lignin pyrolysis by using TG-FTIR analysis. *Journal of Analytical and Applied Pyrolysis*, 82(1), 170-177.
- Lu, Q., Li, W.Z., & Zhu, X.F. (2009). Overview of fuel properties of biomass fast pyrolysis oils. *Energy Conversion Management*, 50, 1376–1383.
- Oramahi, H.A. & Yoshimura, T. (2013). Antifungal and antitermitic activities of wood vinegar from *Vitex pubescens* Vahl. *Journal of Wood Science*, 59(4), 344-350.
- Ruiz de Escudero, I., Banyuls, N., Bel, Y., Maeztu, M., Escriche, B., Muñoz, D. ... Ferré, J. (2014). A screening of five *Bacillus thuringiensis* Vip3A proteins for their activity against lepidopteran pests. *Journal of Invertebrate Pathology*, 117, 51-55.
- Sayed, A.M.M & Behle, R.W. (2017a). Evaluating a dual microbial agent biopesticide with *Bacillus thuringiensis* var. *kurstaki* and *Beauveria bassiana* blastospores. *Biocontrol Science and Technology*, 27(4), 461-474, doi: 10.1080/09583157.2017.1303662
- Sayed, A.M.M. & Behle, R.W. (2017b). Comparing formulations for a mixed-microbial biopesticide with *Bacillus thuringiensis* var. *kurstaki* and *Beauveria bassiana* blastospores. *Archives of Phytopathology and Plant Protection*, 50(15-16), 745-760. doi:10.1080/03235408.2017.1372942

- Sensoz, S., Demiral, I., & Gerçel, H.F. (2006). Olive bagasse (*Olea europea* L.) pyrolysis. *Journal of Bioresource Technology*, 97(3), 429-436.
- Sharma, R.K., Wooten, J.B., Baliga, V.L., Lin, X., Chan, W.G., & Hajaligol, M.R. (2004). Characterization of chars from pyrolysis of lignin. *Journal of Fuel*, 83(11-12), 1469-1482.
- Suqi, L., Caceres, L., Schieck, K., Booker, C.J., McGarvey, B.M., Yeung, K.K.C.... Scott, I. M. (2014). Insecticidal activity of bio-oil from the pyrolysis of straw from *Brassica* spp. *Journal of Agricultural and Food Chemistry*, 62(16), 3610-3618.
- Tiilikkala, K., Lindqvist, I., Hagner, M., Setälä, H., & Perdikis, D. (2011). Use of botanical pesticides in modern plant protection. In: Stoytcheva, M. (Ed.) *Pesticides in the modern world - Pesticides use and management* (pp 259-272). London, UK: InTech. doi: 10.5772/17737
- Wang, D., Zhang, W., Hao, X., Zhou, D. (2013). Transport of biochar particles in saturated granular media: Effects of pyrolysis temperature and particle size. *Environmental Science and Technology*, 47(2), 821-828.
- Warnock, D., Lehmann, J., Kuyper, T., & Rillig, M. (2007). Mycorrhizal responses to biochar in soil – Concepts and mechanisms. *Plant and Soil*, 300, 9-20.
- Wraight, S.P., Ramos, M.E., Avery, P.B., Jaronski, S.T., & Vandenberg, J.D. (2010). Comparative virulence of *Beauveria bassiana* isolates against lepidopteran pests of vegetable crops. *Journal of Invertebrate Pathology*, 103(3), 186-199.
- Yan, X., Wang, X., Han, R., & Qiu, X., (2014). Utilisation of entomopathogenic nematodes, *Heterorhabditis* spp. and *Steinernema* spp., for the control of *Agrotis ipsilon* (Lepidoptera, Noctuidae) in China. *Nematology*, 16(1), 31-40.
- Yang, H., Yan, R., Chen, H., Lee, D.H., & Zheng C. (2007). Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel*, 86(12-13), 1781-1788.
- Yao, Q., Liu, J., Yu, Z., Li, Y., Jin, J., Liu, X., & Wang, G. (2017). Changes of bacterial community compositions after three years of biochar application in a black soil of northeast China. *Applied Soil Ecology*, 113, 11-21.

Insekticidno delovanje bio-ulja i bio-uglja kao proizvoda pirolize i njihovo kombinovanje sa mikrobiološkim agensima za suzbijanje *Agrotis ipsilon* (Lepidoptera: Noctuidae)

REZIME

Tehnologija pirolize za proizvodnju bio-uglja i bio-ulja može se koristiti i kao potencijalna alternativa za proizvodnju biopesticida koji su neodložno potrebni u programima integralne zaštite od štetočina (IPM). Tri komponente bio-ulja: vodena, organska i njihova mešavina, ocenjuvane su pojedinačno i u kombinaciji sa upotrebom tri entomopatogena: bakulovirusom *Agrotis ipsilon* nukleopolihedrovirus (*AgipMNPV*), bakterijom *Bacillus thuringensis* var. *kurstaki* (*Bt*) i gljivicom *Beauveria bassiana* (*Bb*). Delovanje bazne sredine suvog nanosa bio-uglja je proučavano istovremeno sa mikrobiološkim patogenima. Rezultati su pokazali da je organska faza bio-ulja najaktivnija jer je proizvela 100% smrtnosti nakon 24 h, kada su utvrđene srednje vrednosti toksičnosti LC_{50S} (mg/ml). Međutim, frakcije bio-ulja primenjene nezavisno pokazale su tendenciju više smrtnosti kod izloženih larvi nego kombinacija sa mikrobiološkim agensima. Rezultati su takođe pokazali da je smrtnost bila najveća nakon suve primene formulacija sa bio-ugljem na pH 7.1. Zaključak je da ulja proizvedena pirolizom predstavljaju efikasne insekticide, a bio-ugalj bi mogao biti koristan aditiv u proizvodnji i formulaciji biopesticida. Ovaj zanimljiv nalaz promoviše korišćenje jedinjenja iz pirolize, bio-ulja i bio-uglja, kao ekološki prihvatljivu alternativu konvencionalnim pesticidima.

Ključne reči: Proizvodi pirolize; Mikrobiološki agensi; Insekticidno delovanje; *Agrotis ipsilon*