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Impact of tomato wastes pyrolysis liquid against potato whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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Abstract:

Slow pyrolysis of tomato wastes, at temperature of 350 °C results form of Light Pyrolysis Liquid (LPL), which has further separation into aqueous fractions and organic layer. Fourier Transform Infrared spectroscopy (FTIR) was submitted to identify the component of each fraction. It demonstrated the presence of acetic acid, 1-amino-2-propanol, cresol, dimethoxyphenol, pyrogallic and anthracene. Toxicological experiment for every fraction was compared with the commercial formulations of thiamethoxam aganist the 2nd Instar nymphs of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) pots trial. The result demonstrated that there was no significant differences between reduction percentages by using LPL and thiamethoxam. Consequently, biological evaluation test experiment was carried out by measuring reduction percentage of the adult stage of *B. tabaci* on potato plant spunta cv to study the effect of both Light Pyrolysis Liquid and thiamethoxam , the initial kill exceed after three days of application for LPL which reached 84.35% than after one day of application which was 73.9%. For instance, thiamethoxam initial kill reached 74% after three days of application. Subsequently, the residual effect was found to be 85.46 and 73.35 % for LPL and thiamethoxam, respectively. Our results concluded that, it can be used in integrated pest management as biopesticide as it results from plant by- product. Otherwise, there is a continues need for the development of new agrochemical products to provide growers with tools needed to address pest control problems specifically, reducing the risk on resistance development as it has a new mode of action due to presence of mixture compounds.

Introduction

Agricultural wastes have a high contents of lignin and cellulous in addition to some active ingredient (Abou Hussein and Sawan , 2010). Traditionally farmers get rid of these wastes by burning. Only few amounts go in forming composts. Buring procedure causes a serious impact on environment besides the adverse effect on public. Recently, a new trend was arising to utilize the value from these wastes. Slow pyrolysis away to achieve that. The thermal conversion process (conventional pyrolysis) agricultural wastes are slowly heated in the absence of air. The thermal degradation of organic-based materials at slow heating rates (0.1 – 10 ^{0}C/S) and temperatures between 170 – 400 ^{0}C gives biochar and pyrolysis liquids (distillates) (Tiilikkala *et al.*, 2010). Pyrolysis liquid are found in different phases (Yanik *et al*., 2007). Pyrolysis has been used to generate many products since the times of ancient Egypt. Early products were used to caulk boats and embalm and mummify human remains (Murray *et al*., 2014). Furthermore, pyrolysis liquid can be used as biopesticides for pest management (Hossain *et al*., 2013).

Agricultural productivity remains a critical need to address due to the growing population in Egypt that is estimated to reach 120 million people by 2050. There is a demand for increasing food production. Potato (*Solanum tuberosum* L.) are grown and eaten in more countries than any other crop, and in the global economy it is the fourth most important crop after the three cereals maize, rice, and wheat and is one of the most important vegetable crops in Egypt for local consumption and exportation with an annual production of 325 million tons (Stat, 2012). In 2016, Egypt was ranked 14th in the world, with 5.0 million tons of potatoes produced (Faostat, 2016). On the contrary great annual losses have been caused by pests (Metspalu *et al.*, 2001).

The potato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is key pest not only for vegetables but also for agronomic, horticultural, and ornamental crops throughout warm regions of the world (Brown, 1994) White flies, has strong flying capability more than 100 km (Byrne, 1999). In addition , it transmits many types of virues such as the begomoviruses, sweet potato leaf curl virus (SPLCV) and ipomoea leaf curl virus (ILCV), and the ipomovirus Sweet potato mild mottle virus (Valverde *et al*., 2004) .Moreover *M. persicae* also play a key role in the establishment and the dissemination of plant viruses (Boukhris-Bouhachem *et al*., 2017). These pests not only causing losses in crops through feeding on leaves, flowers, or fruit which resulting in reduction in yield but also the high densities can cause irregular ripening disorder in crops which is induced by phloem feeding and toxic saliva (Schuster *et al*., 1996). Furthermore, *B. tabaci* population has a problem of insecticide resistance which are widely diffused so that conventional chemical control doesn’t give the prospect results (Castle *et al*., 2009).Thus, there is a continuing need for the development of new agrochemical products to provide growers with tools needed to address pest control problems. Globally, there is a great need to minimize the environmental risks resulting from pesticides leaching to ground water. The ability of the pyrolysis liquid to target certain agricultural pests could be an asset as a future simple and easy application. The pyrolysis liquid contains a mixture of chemical compounds which have a distinctive odor which can significantly repel pests (Booker *et al*., 2010). It is important to consider the type of biomass component in order to produce a pyrolysis liquid for potential pesticide development.

Therefore, the current work was conducted with the objective of study the possibility of convert farm wastes (tomato wastes) to useful product (pesticide).

Materials and methods

1. Preparation of pyrolysis liquid from tomato wastes:

Dried feedstock of tomato wastes leaves, roots and stems, approximately 100 kg were submitted to pyrolysis using RAUSSI mobile batch retort of the Egyptian-Finnish Agricultural Research Project in Sinai. Slow pyrolysis process was achieved at 350 ^{0}C. The retort was provided with cooling system. The gases and vapors were condensed. The condensables were collected as Pyrolysis Liquid that was left to stand for 24 hours.

2. Separation and extraction of the light pyrolysis liquid:

Analytical process was started with fractionated of Pyrolysis Liquids as two phases. Light Pyrolysis Liquid (LPL) complex chemical composition and precipitated tar at the bottom. To fractionate the LPL liquid–liquid extraction method was followed (Maggi and Delmon, 1994 and Vasalos *et al*., 1994). The pyrolysis liquid components separation was based on polarity (figure 1) was performed in the laboratory of Plant Protection Institute at Ismailia Agriculture research station. The two fractions (LPL and Tar) were separated based on color differences. The organic solvent Dichloromethane (DCM) 100 ml was added to 100 ml LPL to separate organic compounds using separatory funnel. The obtained organic and the remaining compounds were calibrated. Both compounds subjected to partition procedures (Figure, 1).

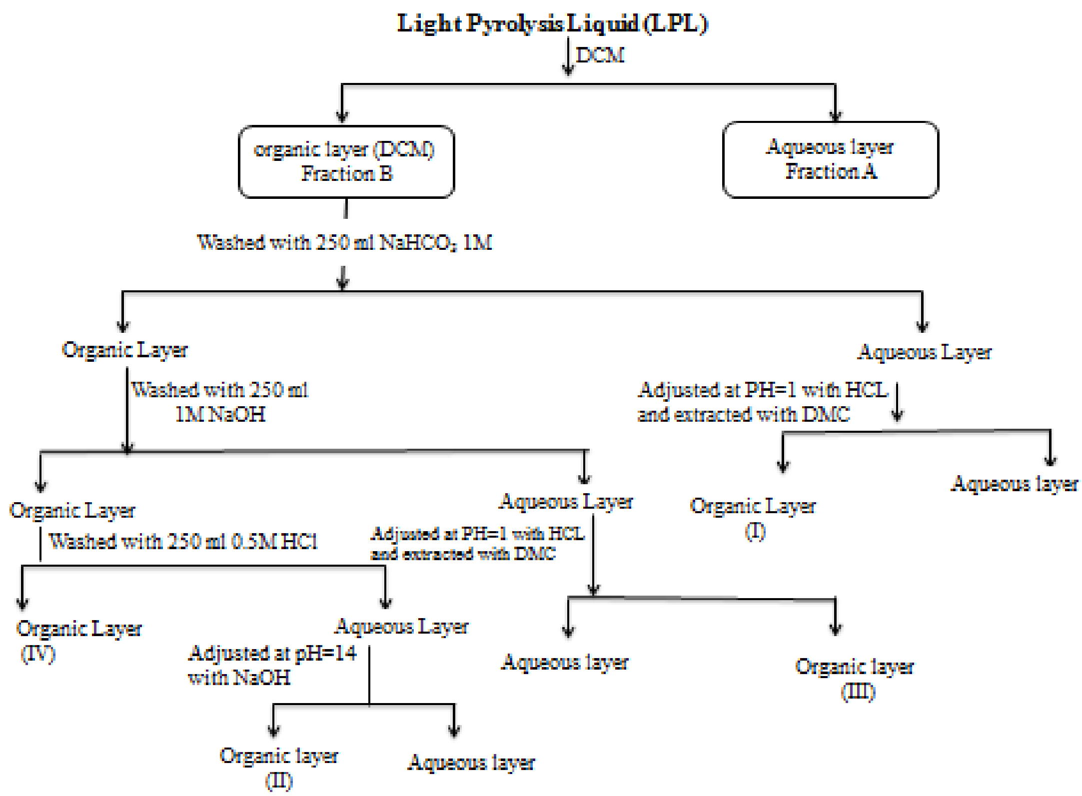


Figure (1) : Liquid-liquid fractionation scheme for Light Pyrolysis Liquid.

LPL were divided into aqueous fractions (Fraction A) and organic layer (Fraction B). Furthermore, the organic layer separated into four fractions .250 ml of $NaHCO_3$ was added to the organic layer which reacted with acids forming two layers (Organic and Aqueous layers). Separatory funnel was employed for separation. 30 ml HCL was added to the aqueous layer to adjust the PH. Then 30 ml solvent DMC was added. The two layers were separated into organic (I) and aqueous layers. 250 ml NaOH was added to the organic layer to adjust the PH. Then 30 ml solvent DMC was added. The two layers were separated into organic and aqueous layers. 30 ml HCL was added to the aqueous layer to adjust the PH. Then 30 ml solvent DMC was added. The two layers were separated into Organic (III) and Aqueous layers. 250 ml HCL was added to the Organic layer to adjust the PH. The two layers were separated into organic (IV) and aqueous layers. 250 ml NaOH was added to the aqueous layer to adjust the PH. The two layers were separated into organic (II) and aqueous layers. Four glass columns chromatography (51 x 5.1 cm) were packed with silica gel to separate each compound from the fraction. Sample from every fraction was injected into the column using different mobile phase. The aqueous solution was dried with sodium disulphate (Na_2S0_4). Each solutions was transferred in a glass bottles to the laboratory of Plant Protection Institute at Ismailia Agriculture research station. Each fraction applied to bioassay to identify the active fraction against pests.

3. **Analysis and characterization of the Light Pyrolysis Liquid:**

Analysis and characterization of the final obtained compounds. The principal components of each was recorded by aid of IR spectroscopy. FT- IR spectroscopy of each sample relied on a Bio-Rad FTIS - 40 model, USA.

4. **Toxicological experiments :**

Potato plants *S. tuberosum* were grown in 15 cm diameter pots filled with a mixture of 1:1 sand and peat moss.

4.1. Maintenance of the strain:

Whitefly *B. tabaci* was collected from potato fields using a mouth aspirator and released on the grown potato plants pots. The strain was reared in muslin cages (0.60 x 0.60 x 1.00 m) .The cage contained four pots each planted with 2 potato plants. The cage was kept in control. Whitefly *B. tabaci* was reared for 2 months away from any contamination at 25 ± 2 °C , 50 ± 5 % RH and a 16-h photoperiod at the laboratory of plant protection institute at Ismailia Agricultural Research Station, Egypt (El-Zahi *et al*., 2017)

4.2. Tested compounds:-

Experiment was conducted to evaluate the efficacy of obtained compounds against the second instar nymphs *B. tabaci* on potato plant.

The following compounds were used throughout this study:

-Pyrolysis liquid fraction A .

-Pyrolysis Liquid fraction B (I ,II , III and VI).

-Light Pyrolysis Liquid (LPL).

-Commercial formulation of thiamethoxam (Thiamex 25% WG , MAC-GmbH, Germany) was tested in at their recommended dose.

The tested compound were sprayed by the aid of knapsack sprayer

4.3. Pots trial:

Whitefly *B. tabaci* was collected from potato strain. The adults could oviposit and put their eggs for 3h. To obtain *B. tabaci* immatures of uniform ages and the adult were removed. The infested leaves were labeled thereafter, *B. tabaci* eggs could develop for 9–10 days to second instar nymphs. Four series concentrations 10, 20, 40 and 80 % were used to determine the toxicity of the tested compounds on the second instar nymph. Mortalities were recorded on the second instar nymphs after 24 hours of application The mortality percentage were corrected (Abbott, 1925). The criteria of mortality is flatted nymphs and easy to remove from the leaf surface with a fine brush (Horowitz *et al*., 1998) .

4.4.Cultivation of potato plants:

Invitro propagation of potato was performed on MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose (MS-3) and 0.8% agar to obtain virus-free material for infection assays. Explants were grown at 22C under a 16/8 h light/dark photo period.Virus-free potato minitubers cv. spunta were used after being harvested and kept at 4°C for periods of 1–6 months before cultivation under screen house to be protected it from insect virus carrier. While growing, the plants need to be protected from insect pests to avoid new disease infections. The soil was sandy loam type. All normal agricultural practices; i.e., irrigation, fertilization, and weed control for growing potato plants were performed. In this experiment, three screen houses with a dimension 30, 9 and 3 meter for length, width and height, respectively were utilized at the Agriculture Research Station, Ismailia. It was ploughed and prepared for planting. Each screen house area was divided into 4 rows with 30 meters long, separated by a gap of 0.5 meters. Spunta cv. was transplanted at distance of 10 cm in rows on 1st February on the two seasons of 2018 and 2019.

4.5.Screen house trail:

To evaluate the efficacy of LPL against the white fly *B. tabaci* (adult) (Mattos *et al*., 2019). The LPL was diluted with water with ratio of 1: 10 , respectively .Plastic containers (50ml) that have a semi open cover were full with the solution. The container were hanged , at a height around 1 - 1.5 meters with distance 3 meter far from each other. The reduction percentages were calculated. The numbers of the adults were recorded after 0*, 1 ,3, 7 and 10 days of application. Another screen house was served as control **(Henderson and Tilton, 1955).**

4.6. Statistical analysis:

Data obtained in both laboratory and screen house experiments were subjected to computerized statistical analysis. Duncan's multiple range tests was used to determine the significant differences between the mean values of the tested material using CoStat system for Windows, Version 6.311.

Results and discussion

1. **Preparation and separation of pyrolysis liquid from tomato wastes:**

The slow pyrolysis process on tomato straw reached 350 $^{\circ}$C in 2 Hours and 45 minutes. The outputs of the cycle were 35.5 Kg biochar and 42 L pyrolysis liquid. With further separation of liquid, we found that about 10 L were precipitated tar and 32 L were Light Pyrolysis Liquid (LPL).The quantitative analysis obtained from liquid–liquid extraction which were used to fractionate the LPL (Figure, 2) was found to be about 20.4 % for aqueous fractions (Fraction A) and 74.4 % for the organic layer (Fraction B). Furthermore, 49.8, 0.9, 14.7 and 9 % for the acidic fraction (I), basic fraction (II), phenolic fraction (III) and hydrocarbon fraction (IV), respectively .

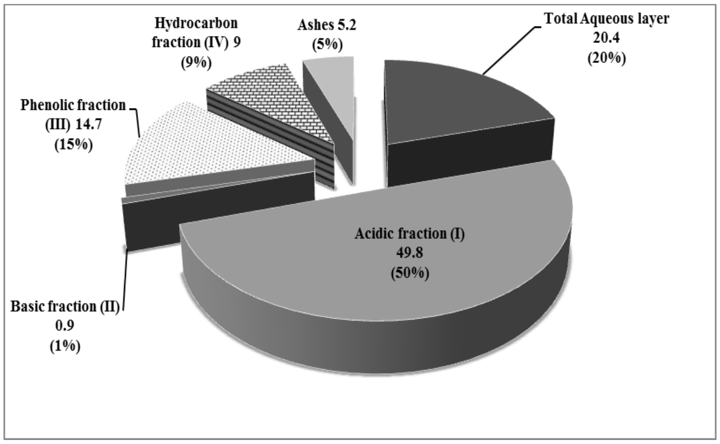
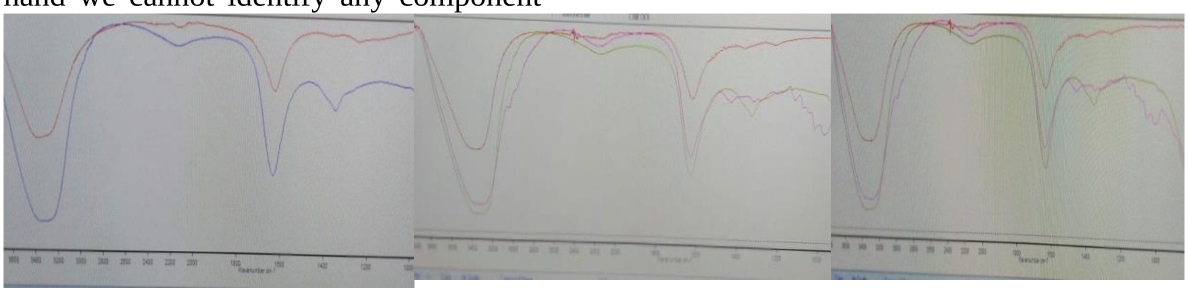


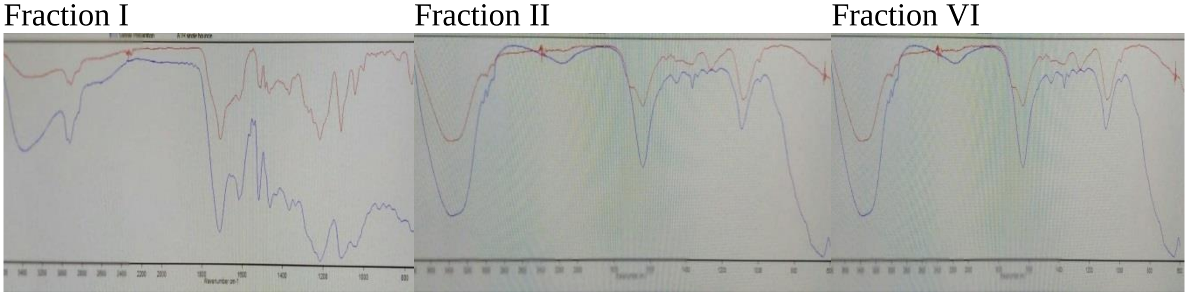
Figure (2): Liquid-liquid fractionation percentage for Light Pyrolysis Liquid.

2. Analysis and characterization of the Light Pyrolysis Liquid:

Fourier Transform Infrared spectroscopy (FTIR) was submitted to identify the component of each fraction automatically only the major compounds. As the result show in Figure (3) on one hand we cannot identify any component from the aqueous layer (fraction A) in which the chart showed to be mysterious, but on the other hand the organic layer (fraction B) we can clearly identify the acidic fraction (I) , basic fraction (II), phenolic fraction (III) and the hydrocarbon fraction (VI).



Fraction I | Fraction II | Fraction VI

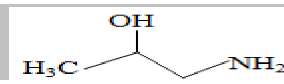
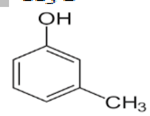
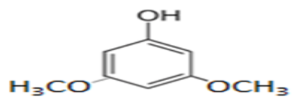
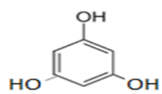
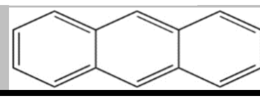


Fraction III

Figure (3): The chart of major components in fraction B by FT- IR spectroscopy.

From the previous chart obtained from FT-IR spectroscopy shown in Figure (3) the peaks automatically identify the presence of acetic acid that found in the acidic fraction (I) besides the presence of 1-amino-2-propanol in basic fraction (II). Likewise, Cresol, Dimethoxyphenol and Pyrogallic that found in phenolic fraction (III). Finally the presence of Anthracene in the hydrocarbon fraction (VI).The six major chemical formula of each compound are shown in Table (1).

Table (1): Characterization of the major components in fraction by FT- IR spectroscopy.

Fractions		Chemical Formula	Chemical Name
Fraction A	**Aqueous Fractions**		----
Fraction B	**Acidic Fraction I**	CH_3-COOH	**Acetic acid**
	Basic Fraction II		**1-amino-2-propanol**
	Phenolic Fraction III		**Cresol**
			**Dimethoxyphenol**
			**Pyrogallic**
	Hydrocarbon fraction VI		**Anthracene**

3 .Toxicological experiments :

The efficacy of the used materials in terms of mortality against the 2nd instar nymph *B. tabaci* on potato plant after 24 hours of application pots trial is shown in Table (2). Data revealed that all used material reduced the mean numbers of the 2nd instar nymph compared with that on control. The increment in concentration and the used materials fluctuate from the mean number of the 2nd instar larva. We observed that the mortality percentage has a limit increase by increasing the concentration for every fraction. On the contrary, using the fractions and the pesticide had resulted in the mortality percentage. Data indicated that the highest percentage of reduction was by using Thiamex 25% WG followed by LPL, while there was no significant difference between them. The usage of fraction A showed 30% mortality, however Fraction B (I, II, III and IV) improved the mortality percentage, which were found to be 67.50, 51.25, 56.25 and 32.50 ,respectively.

Table (2) : Mortality percentage of the 2nd instar nymphs *Bemisia tabaci* pot trial .

Materials		Mortality % 2nd instar larva of whitefly *Bemisia tabaci* with different concentrations±SD			
Concentrations		**10%**	**20%***	**40%***	**80%***
Fraction A		**30.00±0.82**	**35.00±0.82**	**38.75±0.5**	**47.50±1.3**
Fraction B	**I**	**67.50±1.3**	**63.75±1.7**	**62.50±1.7**	**60.00±1.4**
	II	**51.25±0.5**	**56.25±0.9**	**58.75±1.5**	**62.50±1.5**
	III	**56.25±0.9**	**60.00±0.8**	**61.25±0.9**	**63.75±0.9**
	IV	**32.50±1**	**31.25±0.5**	**38.75±0.5**	**42.50±1**
LPL		**87.25±0.9**	**92.50±0.6**	**93.75±0.5**	**93.75±0.5**
Thiamex 25% WG					**90±1.5**
Control					**2.5±0.5**
LSD at 0.05 probability level					**0.5**

***Concentration show phytotoxicity on potato plant.**

Screen house Trail

The experiment was conducted to evaluate the efficacy of 10% LPL and Thiamethoxam against whitefly, *B. tabaci* on potato during 2018 and 2019. Data in Table (3) summarized the assessment of reduction percentage with time (Day). It shows that after ten days of application, the lowest population (1.9/leaf) with reduction percentage of about 85.97 % was observed by using the LPL. On the contrary with the using of Thiamethoxam, the population reached 3.75/leaf with reduction percentage of about 71.69 %. Data also showed that the initial kill increase after three days of application of LPL which reached 84.35% than after one day of application which was 73.9%. For instance, Thiamethoxam initial kill reached 74% after three days of application. Subsequently, the residual effect was found to be 85.46 and 73.35 % for LPL and Thiamethoxam, respectively.

Table (3): Assessment of reduction percentage on the adult stage of whitefly *Bemisia tabaci* by time.

Material		**Mean population of the adult stage whitefly *Bemisia tabaci* / potato leaf (Assessment of Reduction % by Time(Day) after application)**					**Reduction Percentage**	
	Time	0*	1	3	7	10	Initial kill	Residual Effect
LPL		11.50	03.00 (73.9)	02.00 (84.35)	02.00 (84.95)	01.9 (85.97)	84.35	85.46
Thiamethoxam		11.25	03.00 (73.33)	03.25 (74.00)	03.25 (75.00)	03.75 (71.69)	73.33	73.35
Control		11.25	11.25	12.50	13.00	13.25	--	--
LSD at 0.05 probability level								**2.24**

In fact , the Pyrolysis Liquid obtains from agricultural wastes has a unique chemical and physical properties. It can be used in IPM as biopesticide as it results from plant wastes . Otherwise, there is a continus need for the development of new agrochemical products to provide growers with tools needed to address pest control problems Specifically, reducing the risk on resistance development as it has a new mode of action due to presence of mixture compounds. For instance, the pyrolysis liquid has a broader period of application so more life stages can control. In the meantime, there is a demand for increasing food production

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