Volatiles Dynamics on Solid Interface to Model Chemical Cues Release in Tritrophic Interaction

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Abstract. Phenomena of triptrophic interaction are well known in the agricultural area. Some specific volatiles is released from host plants after the infestation of pest insects, to attract parasitoids. Volatiles are formed during the biochemical process before released to open air. The phenomena of volatiles adsorption and desorption were investigated using spectroscopic methods. The model material was biosilica-nanocellulose which is porous and made by sol-gel processing and represented the wounded tissue plant in the tritrophic interaction. Some similar hydrocarbons from the previous study were adsorbed onto the surface and investigated. Some UV-visible and infrared spectra of adsorbed chemicals were recorded while the released gasses were captured and separated in the gas chromatography column. The result showed qualitatively molecular attachment into polar surfaces, and gas chromatography-mass spectroscopy indirectly determines the molecule desorbed from the solid interface and its pattern.

Keywords: Volatile dynamics, solid interface, triptrophic interaction, polar surface.

1. Introduction

Tritrophic interaction is a common mechanism used by plants in a self-defense situation [1–4]. The interaction between host-plant – herbivore- parasitoid in such condition involves certain chemical cues, which are biosynthesized and released as some volatile compounds. One model of this tritrophic interaction has been explored and rice plant attacked by brown plant-hopper (BPH) was explored. The chemical cues released attracted Anangrus Nilaparvatae parasitoid, and they consisted of mostly long-chained hydrocarbons as well as alcohols and esters [4]. The infested paddy plant was undergoing soxhlet extraction using polar and non-polar solvents and the results were analyzed by gas chromatography and mass spectrometry. Some chemicals were increased in dominance, but there are also new compounds appear and some disappear in the extract after infestation. The result was quite persistent and also in good agreement with other exploration of rice from the other region of Asia [3, [5,6].

The behavior of insects wounding plant leaves-tissue for egg deposition was already explored and described [1]. The insects would choose the best place for egg-laying but this process was also correlated with the chemical cues from the plants as well as with climate and working hour of the insects. The egg-
induced changes to the biochemistry of the plant and as the result, primary and secondary metabolites of the plants would also change. The chemicals from the plants act also to preserve the eggs and influence the quality of insect egg as well as embryo development. However, the real biochemistry routes investigation would need more depth in laboratory experiments. In fact, this phenomenon is becoming more interesting as human being need to imitate the nature to take the control in agriculture work.

To make the story shorter, investigation on chemical cues involved, some extraction method was done and the content was determined by gas chromatography and mass spectrometry [2,3]. This job was clear and in accordance with another result from the Asian region. On the other hand, for bio-control purposes, there were some attempts to make such system for fabrication. A system with similar wounded-moist leaves releasing chemicals is under consideration. A biomaterial from silica and cellulose was under investigation since the structure is porous and can be made humid and then some similar hydrocarbon or other volatile compounds can be made released from the interface. In this case, similar wounded leaves are on the attempt.

The study of the released volatiles from wounded leaves has not been investigated so far. The material of the leaves can be cellulose, lignin and many other organic compounds covered by kind of paraffin to adjust the moist besides other functions for defense. When it is wounded by herbivores, some holes are created and volatiles is released due to abiotic stress. This type of situation is to be imitated while the material themselves are designed from the laboratory. Combination of silica and cellulose were chosen due to their properties and the similarity with leaves system. Raw materials were chosen from biomaterial too, those are rice husk ash as the source of silica and hydrolyzed nata de coco as the source of cellulose.

Silica was extracted from rice husk ash and gelled by sol-gel processing, during which nano-cellulose was added to alter the surface [4]. Originally the silica was extracted from rice husk ash by sol-gel processing [5]. Nano-cellulose was obtained by hydrolysis in the sulfuric acid catalyst. The cellulose itself was taken from bacterial cellulose "nata de coco" which was made with the aid of Acetobacter xylinum [6,7]. This cellulose is relatively pure and ready to hydrolyze to cut the long carbon chains. Working with cellulose, there are more possibilities to explore since modification with the cellulose can be done for various applications [8–11]. This type of material has more applications as adsorbent and still more exploration would be done related to solid-gas and solid-liquid interface. Therefore some applications of cellulose surface are for adsorbents and in this opportunity the silica and hydrolyzed cellulose during gelling in the sol-gel processing. This method of material for adsorbent was registered as Indonesian patent in 2017 with the register number P00201508272. The imitated wounded leaves from silica-cellulose were then used to observe hydrocarbon release. Some similar chemical compounds were adsorbent onto the surface and the amount released as well as the retention time were tried in the laboratory as well as in olfactometer systems. This type of formulation was also registered as an Indonesian patent. The big aim of this project was to make similar attractant from the similar adsorbent (silica and cellulose undergo gelling together) and similar synthetic components of volatiles added intentionally. The study in this experiments covers the dynamics of those molecules in the solid-gas interface as can be approached using FTIR and GCMS instruments.

2. Experimental Methods
In this part, there were two sections, the formulation of bio-attractant from silica-cellulose material and the spectroscopy study using infrared spectroscopy for the adsorbed attractant and gas-chromatography-mass spectrometry for the released gas separation and identification. One series of mass measurements were done to get information about attractant given off the surface to the air especially during the earlier stages of the making. The third part is about bioassay, to prove the volatiles given off by attracting Anangrus Nilaparvatae parasitoid to the source of a synthetic odor.

The chemical used in this study were obtained from Sigma Aldrich and E. Merck. Most of the solutions needed were made using distilled water. FT-IR measurement used IRPrestige-21 from
Shimadzu and GC/MS experiments were done in QP2010Plus from Shimadzu. Scanning Electron Microscope used was the one from FEI Type Inspec S50. All bench activities used pyrex glassware.

Materials for adsorbent was made of rice-husk ash as the source of silica, according to Kalapathy [12] with some modification. The extraction was by sol-gel processing and some hydrolized cellulose were added during the gelation process for the in-situ incorporation. The method has been registered in Indonesian Patent (2nd December 2015, No. P00201508272). The bio-attractant formulation was done using some hydrocarbon compounds namely heptane, hexadecane, and n-hexane was used as a standard. The other system used 1-octanol, butyl acetate, and hexatriacontane. The third system used the same compounds but the adsorbent contained one surfactant (n-CTMABr) to shift the surface properties and to make the material homogeneous in the pore system. The real formulation method of bio-attractant was also registered in Indonesian Patent with registration number P00201603970, 16th June 2016. The bio-attractant were put in closed vials for spectroscopy measurement. The mass-reduction measurement was done immediately after formulation, for the second and third systems, and the vials were kept open, the same as in the real situation with wounded leaves. FTIR experiments were run at the attractant formula together with the adsorbent as a solid sample, and GC/MS were done for the released gasses after formulation using a gas-tight syringe. The gasses in closed vials were taken by tight syringe gas prior to injection. Bioassay for the bio-attractant was done but would be reported elsewhere.

3. Results and Discussion

There are several sections on this result and discussion. The first section will be discussed the materials for adsorbent, which were used for the bio-attractant formulation by adding some chemicals similar to kairomones compounds extracted before [2]. The appearance of this adsorbent can be found in Figure 1 (a) and the SEM picture can be seen from the magnification of 100,000 times (Figure 1.b). The white powder of silica-cellulose is actually a porous medium, and according to the SEM picture below, the diameter of particles is around 50 nm. This material can be categorized nanomaterial since the diameter, as well as the dimension of porosity, is below 100 nm. This material is also hydrophilic due to polar functional groups on the surface, which come from the silanols and hydroxyl groups of the cellulose. The polar porous surface can retain small molecules, and therefore they can release the gaseous particles slowly and step by step. This is the desirable property of a good releaser so that the bio-attractant can be effective after sometimes. The silica surface itself tends to be too polar that retains the molecules stronger. The presence of cellulose somehow reduces the polarity and the material can be a good and effective releaser.

![Figure 1](image-url)

**Figure 1.** The appearance of silica-cellulose adsorbent after drying (a) and the 100,000x magnification of the material (b).
The white powdered material in Figure 1(a) shows no sign of porosity. Only by big enough magnification can reveal the voids in between particles. The texture of the surface cannot be assigned clearly from the SEM picture. However, the homogeneous particles and porosity can be claimed from the rough observation. The silica particles mixed well with cellulose as globular particles altogether. The system itself is a non-conducting material so that the measurement can be done after a relatively thick coating.

The formulation of bio-attractant was actually done to mimic the wounded leaves due to oviposition of insect pest. Under abiotic stress, some volatiles as developed by biochemistry processes of the plants is released to air to attract the parasitoid of the pest. The volatiles is specific and undergoes complex biochemistry routes of synthesis from jasmonates and its derivates [13,14]. The composition of volatiles was already done in similar ways, namely extraction and identification in the past [2,3,15]. In this case, silica-cellulose adsorbent resembles the leaves tissue, which is also humid and the similar volatile compounds were added to the adsorbent and were released and observed (Figure 2a). The mechanism for releasing volatiles was investigated by FTIR and GC/MS.

![Figure 2](image)

(a) (b)

**Figure 2.** Formulation of bio-attractant with a silica-cellulose adsorbent (a) and mass reduction measurement (b) for 0-1500 minutes.

The mass measurement was done to observe the volatiles release process (Figure 2b). The original mass dropped significantly during the early hour and leveling off after sometimes. However, not all the adsorbed molecules went away, there were some on the surface in equilibrium with outer water molecules as well as other gasses on air. The mass did not change significantly anymore, the up and down in a long time showed the equilibrium on the surface. Moreover, in the FTIR spectra, up to the day-8, the sign of functional groups of the volatiles were still present. The gas released also appeared in GC/MS analysis up to day -10. The observation and analysis of adsorbates, as well as gasses released, would be discussed below.
From the figure above it can be seen that adsorbent material, both with and without adsorbed hydrocarbons give different patterns compared to the liquid hydrocarbons (light blue spectrum). The broad signals indicate restricted vibrations on surfaces and the peaks are broader. However, it gives the signal of hydroxyl groups from silanols (\(\equiv\text{Si-OH}\)) as well as much hydroxyl of cellulose from the stretching vibration around 3600-3200 cm\(^{-1}\) (dark blue spectrum in Figure 3). The spectrum of hydrocarbon mixture indicates clearly the symmetric and asymmetric \(-\text{C-H}\) stretching (around averaged at 2900 cm\(^{-1}\)), \(-\text{CH}_2-\) and \(\text{C-CH}_3\) bending (1471 cm\(^{-1}\) and 1381 cm\(^{-1}\)) of all hydrocarbons. The band around 725 cm\(^{-1}\) looks strong for the bending vibration of the \(-(\text{CH}_2)_{4-8}\) units for all hexane, heptane, and hexadecane. The character of this hydrocarbons still appears after they are absorbed in the surface of silica-cellulose. The detail inside the circles indicates the same components on the surface, as they remain on the surface even though the molecules release themselves after sometimes. The bands broadened themselves since the vibrational modes are now more restricted on polar surface. The signals weaken after 8 days of exposure. This is the first system in this report.

The second system used one long-chained primary alcohol (\(1\)-octanol), one branched ester (butyl-acetate) as one fresh odor source in plants, and also one long chained hydrocarbon (hexatriacontane). These three types of organic compounds have different polarity and also the tendency of retention and interaction on the polar surface. However, it is difficult to distinguish which interaction is which and what appears in the spectrum would be the net result of so many possibilities in interactions.

The additional functional groups on this system can be seen in the blue spectrum above. The primary hydroxyl group from \(1\)-octanol can be seen from the stretching vibration which appears broad due to hydrogen bonding and also together with hydrogen bonding from the surface water after adsorption. Additional small peaks appear in the fingerprint area are from the character of acetate esters, which in turn are not seen in the adsorbed phase. However, the long-chained hexatriacontane cannot be avoided even after adsorption. This is only a qualitative assignment, there is no way to calculate the adsorbates quantitatively using the simple infrared spectrometer.

The third system in this series used surfactant on the surface. \(n\)-Cetyl Trimethyl Ammonium Bromide (Figure 5 a-b) is one cationic surfactant that is used mainly to alter the surface property. In solution, it is readily ionized. In making materials, surfactants are used as templates in the gelation stage [16]. The gelling process to make porous material can be followed in situ by NMR method[17] besides FTIR discussed previously. The negative and positive ends of the long chained compound can make micelle according to the surface and space-configuration so that the main charge, as well as its distribution, shifted. However, the appearance of FTIR spectrum of silica-cellulose is more or less the same as the previous adsorbents since after the gelation the adsorbents were washed with distilled water. The small amount of surfactant is not revealed in the spectrum even though it might alter the surface properties and make the porous system homogeneous.
Figure 4. Combined IR spectrum of silica-cellulose adsorbent (grey), (1:1:1) mixture of n-hexane, n-heptane, n-hexadecane (light blue), adsorbed mixture onto adsorbent (green) after twelve days exposure.

Within the porous medium, the complexity increases and possible interaction with adsorbate compounds cannot be assign from FTIR spectrum. When chemical bonding with the surface is not occurring, the interactions between anything on the surface would be reversible. That is why the volatile compounds can be released into the air and compounds in solution undergo surface dynamics together with surface solvent molecules.

Figure 5. The molecular structure of n-CTAB (a) and one possible interaction in silica surface (b) as given in Holmes et.al. [16].

Gas analysis attempt was done by separating in silica derivate column and analyze the gas by mass spectrometry. The original compounds were done before the adsorbed on the silica-cellulose surface. In Figure 6 we can see the appearance of adsorbed hexane, heptane, and hexadecane cannot be separated well since they are strongly bonded to the surface. Since the three peaks are so broad, they cannot be resolved well in the similar retention time. This, however, cannot explain the retention mechanism on the surface.
Equilibration in porous medium takes sometimes. There was something interesting in the chromatogram of the formulation after one day of exposure. There were some gasses appear in longer retention time even though the assignment from Wiley library is not really clear. However, due to the inhomogeneity of the sample, some analysis must be done. The similar hydrocarbons have almost the same properties since the elements are only carbon and hydrogen. That is how the mass spectrum of the longer retained components was not clear. The unresolved components in earlier retention time (around 2.5 minutes) shows the strong interaction with the surface in the column. Certainly, there was a similar interaction with the surface column which is made of more silica. As we know silica retails more and during elution occurred non-equilibrium mass transfer[18]. Some part of the gas would still retain but due to strong eluent gas flow then some part of the components appeared later (Figure 6.b). Of course, it is not the best condition for the separation and analysis. Also as a consequence, Wiley 8 library still suggested some long-chained hydrocarbons for the peak but only similar with hydrocarbon used, not exactly matched the same as supposed to be.

Analogous to this separation and longer retention time, the investigation of small molecule mobility is a porous medium for the partially filled porous system has been investigated using NMR relaxation and diffusion experiments [19–21]. A strange kinetic occurring as a consequence of surface diffusion has been thoroughly discussed in some paper about NMR methods[22]. Before appeared in longer retention time, the molecules undergo complex behavior related to the affinity of molecules unto the surface. Silica cellulose adsorbent is less polar than silica only so that the gasses were released easier. During separation, the gasses behaved differently.

For the second and third system, the presence of n-CTABr surfactant was also in the adsorbent system, there came the same tendency. In the similar place, comes some unresolved peaks, for the newly made formulation. After some time of equilibration, the unresolved peaks decrease in intensity but appeared more peaks with longer retention times (Figure 7). There was interesting that the number of gasses from the adsorbent made using surfactant appeared more than silica-cellulose only. Since more molecules released from the interface, the amount of volatiles after 5 days of exposure remained less from the surfactant adsorbent. This might due to the more homogeneous matrix of silica while it was gelled with surfactant template [23]. However, after 10 days appeared more volatiles from the surfactant adsorbent compared to the second system. In this case, the system of surfactant adsorbent retained more volatiles in equilibrium, might be related to polar functional groups they have (alcohol, esters, and also hydrocarbon).
Figure 7. The chromatogram of adsorbed 1-octanol, hexatriacontane, and butyl acetate in silica-cellulose made without surfactant (black) and adsorbed mixture on silica-cellulose made with surfactant (pink) from RTX-5 column, immediately after formulation (a) and after 5 days of exposure (b).

After long days of exposure, the unresolved peaks disappeared but the peaks in longer retention time appear clearly (Figure 8). This indicated the ability of the interface to hold the volatile compounds to be released after some time. This is a good property of a bio-attractant so that it still shows effective in use after a long time.

Figure 8. The chromatogram of adsorbed 1-octanol, hexatriacontane, and butyl acetate in silica-cellulose made without surfactant (black) and adsorbed mixture on silica-cellulose made with surfactant (pink) from RTX-5 column, 10 days of exposure.

The presence of gasses must be studied further using bioassay, to see the effect as bioattractant for the parasitoid. The assignment using Wiley 8 database would not explain the real gasses appear in the longer retention times since the similarity index (SI) would not be 100%. Further mass spectrometry must be done to ensure the assignment using the MS database. However, the best method to further investigate the gasses is bioassay using the real parasitoid in olfactometry or in the field, as already did for the extract of infested paddy plant [2,3].

4. Conclusion
The dynamics on the solid-gas interface was followed by mass-reduction experiments, and gas-chromatography-mass spectrometry FTIR spectra described the presence of bio-attractant components in the surface. GC/MS shows the released volatiles from the surface after some certain time retention on surfaces. Polar compounds behave different compared to non-polar hydrocarbons as qualitatively seen in the IR spectra and gas chromatograms. Mass reduction showed the release of gasses from the beginning until equilibration. However, since the original intention of this research is for modeling the tritrophic interaction, the further attempt has to be bioassay using real parasitoid insects. Further research would also deal with surfactant effect in releasing the volatiles or other adsorbates on interfaces in the
more comprehensive discussion. The changing gases released in longer retention time must be clearly explained.

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