Journal of Plant Studies; Vol. 6, No. 1; 2017
ISSN 1927-0461 E-ISSN 1927-047X
Published by Canadian Center of Science and Education

Impact of Different Environmental Stimuli on the Release of 1-MCP from Boron-MCP Complexes

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Received: September 21, 2016 Accepted: October 25, 2016 Online Published: November 29, 2016

doi:10.5539/jps.v6n1p46 URL: http://dx.doi.org/10.5539/jps.v6n1p46

Abstract

In our previous report, boron derivatives of methylene cyclopropane complexes (B-MCP) were developed to stabilize the gaseous 1-MCP (1-methylcyclopropene), a commercial plant growth regulator, for eventual release in open crop fields when under humid conditions or in contact with water. To meet the requirements of various end-use applications, B-MCP compounds that can release 1-MCP at different rates for different time periods are demanded. In this paper, we examined the impact of different environmental stimuli such as humidity, surface area, water pH and water volume on the release kinetics of 1-MCP from B-MCP compounds of various chemical structures. The results showed that the release of 1-MCP from B-MCP compounds can be tailored by altering the architectures and chemical natures of the two short-chain moieties attached to the boron atom and thus either the electronic affinity or the hydrophobic or hydrophilic properties or both.

Keywords: boron derivatives of methylene cyclopropane, B-MCP, open field application, plant growth regulator

1. Introduction

Physiological studies of plants reveal that diffusion of ethylene into and out of plant tissue is responsible for regulating various growth and developmental activities such as ripening of fruits (Yang, 1995; Alexander & Grierson, 2002; Atta-Aly & Brecht, 2000; Klee, 2002; Fan, Blankenship & Mattheis 1999; Fan & Mattheis, 2000), opening of flowers, and senescence (Abeles, Morgan & Saltveit, 1992). However, ethylene also is responsible for the factors that cause defects in product quality or premature death such as loss of cellular turgor and chlorophyll, pigment degradation, irregular opening, yellowing or shedding of flowers and leaves (Serek, Woltering, Sisler, Frello & Sriskandarajah, 2006). 1-Methylcyclopropene (1-MCP), a cyclic olefin used commercially as a synthetic plant growth regulator, is applied onto post-harvest agricultural products such as fruits (including climacteric and non-climacteric), flowers and vegetables to retard their ripening by working as a preservative and thus prolong their shelf-lives (Sisler & Serek, 2003; Blankenship & Dole, 2003). Because of structural similarity to the natural plant hormone, 1-MCP works as a nontoxic antagonist of ethylene, interacting with and then covalently binding to ethylene receptors. (Sisler & Serek, 1997; Blankenship & Dole, 2003; Watkins, 2006; Sisler, Grichko & Serek, 2006). The affinity of 1-MCP to the ethylene receptors is ten times greater than that of ethylene (Watkins, 2006), which means a low concentration of 1-MCP is sufficient for application to horticultural products. 1-MCP completely blocks the absorption of ethylene and its hormonal action (Serek, Tamari, Sisler & Borochov, 1995; Sisler & Serek, 2003; Blankenship & Dole, 2003) for a certain time (Figure 1) until all 1-MCP diffuses from the binding sites or new ethylene receptors develop (Bayer, 1976a, 1976b, 1978; Veen, 1983).

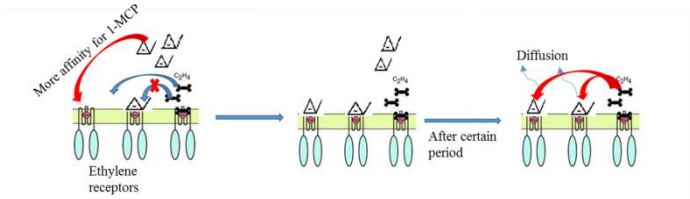

Figure 1. Competition between 1-MCP and Ethylene in blocking ethylene receptors and reopening of receptors for ethylene after diffusion of 1-MCP

Under environmental stresses, plants tend to produce more ethylene, resulting in premature opening or early ripening (Chow & McCourt, 2006; De Paepe & Van der Straeten, 2005). By this mechanism, extreme weather, such as drought, can cause yield losses of grain crops, such as corn, wheat, and rice, etc. In this case, application of 1-MCP in fields is expected to play a role in yield protection as a pre-harvest treatment (Kawakami, Oosterhuis & Snider, 2010). The use of 1-MCP in an open field indicates a huge potential, since about 45% of the total world's agricultural lands are subjected to continuous or frequent drought conditions (Bot, Nachtergaele & Young, 2000).

1-MCP is an odorless gas at ambient conditions (b.p. 9-12 ^{o}C), which makes it difficult to handle in an open environment. At present, there is no technology available for application of 1-MCP in open fields conveniently and effectively. However, the reported B-MCP analog complexes (Sarker, Fan & Liu, 2015; Sarker, Tomasula & Liu, 2016) (Figure 2) have shown significant potential to be directly used in open fields by releasing 1-MCP over a long time period depending on their individual structures. The effectiveness of these complexes to inhibit tomato ripening was demonstrated in an open, laboratory environment and reported in our earlier publication (Sarker et al., 2016).

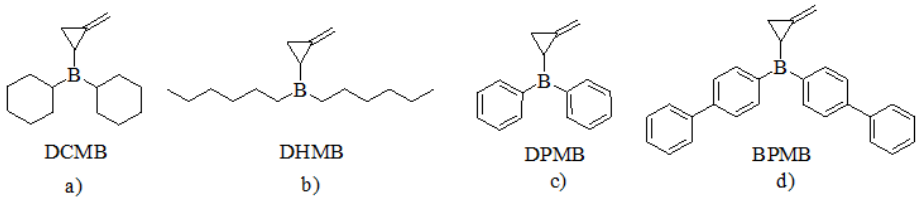

Figure 2. B-MCP derivatives: a) **DCMB**, Dicyclohexyl-(2-methylene cyclopropyl)-borane; b) **DHMB**, Dihexyl-(2-methylene cyclopropyl)-borane; c) **DPMB**, Diphenyl-(2-methylene cyclopropyl)-borane; d) **BPMB**, Bis-biphenyl-4-yl-(2-methylene cyclopropyl)-borane

The chemistry involved in release of 1-MCP from a B-MCP analog is controlled by hydrolysis reaction (Sarker et al., 2015) and the rate of this reaction mainly depends on the chemical and physical properties of the moieties attached to the central boron atom. Therefore, 1-MCP is found to be released at different rates from different complexes which are useful depending on the application. For practical application in an open field, the flexibility to tailor the structure of B-MCP complex is important for having the 1-MCP release at the rate as demanded under different environmental conditions, such as temperature, humidity, wetted area, and pH; e.g. To have a better understanding of the interdependency of the release of 1-MCP on environmental stimuli and the structure of B-MCP, in this paper, we compared the release pattern of 1-MCP of two newly synthesized boron compounds, **BNMB**, Bis-naphthalen-1-yl-(2-methylene cyclopropyl)-borane and **BPNMB**, Bis-phenanthren-9-yl-(2-methylene cyclopropyl)-borane **(**Figure 3) with **DCMB**, Dicyclohexyl-(2-methylene cyclopropyl)-borane and **BPMB**, Bis-biphenyl-4-yl-(2-methylene cyclopropyl)-borane (Figure 2) that was reported previously (Sarker et al., 2016).

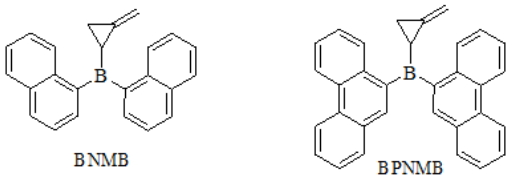

Figure 3. B-MCP derivatives: **BNMB**, Bis-naphthalen-1-yl-(2-methylene cyclopropyl)-borane and **BPNMB**, Bis-phenanthren-9-yl-(2-methylene cyclopropyl)-borane

2. Materials and Methods

2.1 Chemicals

All the chemicals and solvents used for synthetic purposes were purchased from Sigma-Aldrich unless otherwise stated. All solvents used were of HPLC grade and moisture dry.

*2.2 Syntheses of Complexes **BNMB** (13) and **BPNMB** (14) (Figure 4)*

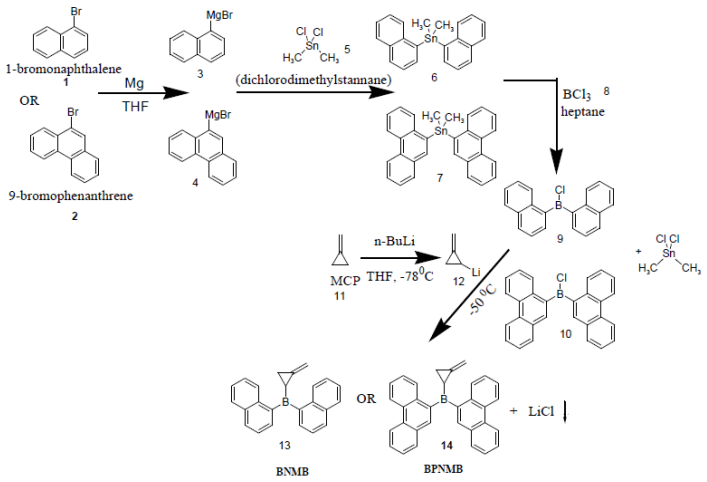

Figure 4. Synthetic scheme of complexes **BNMB** and **BPNMB**

BNMB and **BPNMB** were prepared by the reaction of bis-naphthalen-1-yl-chloro-borane (9) and bis-phenanthren-9-yl-chloro-borane (10) respectively with lithiated methylenecyclopropane (12), whereas, bis-naphthalen-1-yl-chloro-borane and bis-phenanthren-9-yl-chloro-borane were previously made from the reaction of bis-naphthalen-1-yl-dimethyl-stannane (6) and bis-phenanthren-9-yl-dimethyl-stannane (7) respectively with boron trichloride (8) in heptane. Bis-naphthalen-1-yl-dimethyl-stannane and Bis-phenanthren-9-yl-dimethyl-stannane were synthesized by the reaction of dichlorodimethylstannane (5) with Grignard reagents of 1-bromonaphthalene (1) and 9-bromophenanthrene respectively (2). Methylenecyclopropane (MCP) (11) was prepared (not shown in Figure 4) from the reaction between Potassium-bis(trimethylsilyl)-amide and methallylchloride (Binger, Brinkmann & Wedemann, 2002).

2.2.1 Synthesis of **BNMB** Complex (13)

2.2.1a Synthetic Procedure of Bis-naphthalen-1-yl-dimethyl-stannane (6)

An oven-dried 200 mL Schlenk flask cooled under argon was charged with 2.4 g (100 mmol) Mg turnings and 60 ml of anhydrous THF. To the mixture, 14.7 g (9.93 mL, 71 mmol) of 1-bromonaphthalene (1) was added slowly. The reaction mixture was stirred at room temperature for 30 min before it was refluxed for 2 h then cooled at room temperature through Argon flow. The freshly prepared Grignard reagent (3) was transferred via cannula into a 200 mL 2-neck flask equipped with a condenser and cooled at 0 ℃. A solution of dichlorodimethylstannane (5) (7.5 g, 34.1 mmol) in 15 mL of dry THF was added into the 2-neck flask via a cannula. The reaction mixture was refluxed for 3 h then stirred at room temperature for 16 h. After the reaction the solution was cooled at 0 ℃, treated little by little with a total 15 mL of saturated NH_4Cl solution and extracted with dichloromethane in a separatory funnel. The organic layer was washed three times with a total of 250 mL water followed by quenching with brine solution, then dried with Na_2SO_4, concentrated in vacuo to give crude product mixed with naphthalene. The crude product was separated with 100% hexane through flash silica column chromatography. Two fractions were collected: mostly naphthalene in the 1st fraction and pure product in the 2nd fraction. The 2nd fraction was concentrated in vacuo and dried under vacuum to obtain 11.13 g (27.6 mmol) white solid of Bis-naphthalen-1-yl-dimethyl-stannane (6). Product yield was found to be 80.94%. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.82 (3H, s), 7.36 - 7.55 (3H, m), 7.71 (1H, d, J = 6.42 Hz), 7.89 (3H, d, J = 7.55 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ - 7.7, 125.4, 125.5, 125.9, 128.87, 129.04, 129.87, 133.65, 135.5, 138.43, 141.16.

2.2.1b Synthetic Procedure of Bis-naphthalen-1-yl-chloro-borane (9)

An oven dried 250 mL thick-walled flask with Teflon screw cap cooled under argon was charged with 5 g (12.4

mmol) of Bis-naphthalen-1-yl-dimethyl-stannane (6), 40 mL of anhydrous heptane and 13 mL of 1M boron trichloride solution (8) in heptane (13 mmol) in a nitrogen saturated glove box. The mixture was stirred for 30 minutes at room temperature and then heated at 120 ℃ for 96 h. After the reaction, the solution was cooled and subjected to vacuum filtration under nitrogen in the glove box to remove dichlorodimethylstannane (5) as a solid. The solid was washed with the least amount of anhydrous heptane and the collected heptane solution was concentrated in a rotary evaporator. The rest of dichlorodimethylstanane was removed by a sublimation technique at least 3 times and dried under vacuum to obtain pure 3.57 g (11.88 mmol) ash colored solid material of Bis-naphthalen-1-yl-chloro-borane (9). Product yield was 95.8%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 (1H, t, J = 7.60 Hz), 7.48 (1H, t, J = 7.59 Hz), 7.54 (1H, t, J = 7.59 Hz), 7.91 (1H, d, J = 7.94 Hz), 7.98 (1H, d, J = 6.90 Hz), 8.06 (1H, d, J = 7.94 Hz), 8.10 (1H, d, J = 8.29 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 125.85, 127.16, 127.48, 127.66, 128.93, 130.47, 132.72, 135.69, 136.51, 137.71; ^{11}B NMR (128 MHz, $CDCl_3$): δ 64.9.

2.2.1c Synthetic Procedure of Bis-naphthalen-1-yl-(2-methylenecyclopropyl)-borane (13), **BNMB**

An oven-dried 100 mL Schlenk flask cooled at -78 ℃ was charged with 1 mL MCP (11) (0.82 g, 15.2 mmol) in 15 mL of anhydrous THF. To the solution, 5.5 mL of 2.5M n-BuLi in hexane (13.75 mmol) was added slowly and stirred at room temperature for 3 h. The mixture was cooled at -50 ℃ and 4.54 g (15.1 mmol) of bis-naphthalen-1-yl-chloro-borane (9) dissolved in 15 mL of dry THF was added slowly, over 15 min, via a cannula. The reaction mixture was stirred at room temperature for 24 h. The solution was filtered under Argon to remove salt and concentrated in vacuo. The solid was dissolved in 20 mL of anhydrous dichloromethane and filtered with a syringe filter to get a clear solution. The solution was concentrated in vacuo and dried under vacuum to obtain 3.1 g (9.74 mmol) solid of **BNMB** (13). Product yield 64.5%. ^{11}B NMR (128 MHz, $CDCl_3$): δ 48.3.

2.2.2 Synthesis of **BPNMB** Complex

2.2.2a Synthetic Procedure of Bis-phenanthren-9-yl-dimethyl-stannane (7)

An oven-dried 200 mL Schlenk flask cooled under argon was charged with 1.9 g Mg turnings (78.2 mmol) and 55 mL of anhydrous THF. To the mixture, 12.6 g (49 mmol) of 9-bromphenanthrene (2) dissolved in 15 mL of anhydrous THF was added slowly via cannula. The reaction mixture was stirred at room temperature for 30 min before it was refluxed for 2 h then cooled at room temperature through argon flow. The freshly prepared Grignard reagent (4) was transferred via cannula into a 200 mL 2-neck flask equipped with a condenser over an ice bath. A solution of dichlorodimethylstannane (5) (5 g, 22.76 mmol) in 10 mL of dry THF was added into the 2-neck flask via a cannula. The mixture was refluxed for 3 h then stirred at room temperature for 16 h. After the reaction, the solution was cooled at 0 ℃, treated little by little with a total of 10 mL saturated NH_4Cl solution and extracted with dichloromethane in a separatory funnel. The organic layer was washed three times with a total of 150 mL water followed by quenching with brine solution, then dried with Na_2SO_4, concentrated in vacuo to give a crude product mixed with phenanthrene. The crude product dissolved in least amount (~30 mL) of CH_2Cl_2 was added 50 mL of hexane to get precipitation. The precipitate was filtered under vacuum and checked for the purity with thin layer chromatography (TLC) in 100% hexane as mobile phase. For any further impurity, the solid was heated at 80 ^{o}C with 30 mL of hexane and filtered immediately to obtain pure 7.4 g (14.68 mmol) solid of Bis-phenanthren-9-yl-dimethyl-stannane (7). Product yield 64.5%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (3H, s), 7.49 (1H, t, J = 7.52 Hz), 7.54 – 7.73 (3H, m), 7.80 (1H, d, J = 7.52 Hz), 7.96 (1H, d, J = 7.82 Hz), 8.01 (1H, s), 8.72 (1H, d, J = 8.12 Hz), 8.76 (1H, d, J = 8.12 Hz).

2.2.2b Synthetic Procedure of Bis-phenanthren-9-yl-chloro-borane (10)

An oven dried 250 mL thick-walled flask with Teflon screw cap cooled under argon was charged with 6.5 g (12.9 mmol) of Bis-phenanthren-9-yl-dimethyl-stannane (7), 15 mL of anhydrous heptane and 13.5 mL (13.5 mmol) of 1M boron trichloride solution in heptane (8) in a nitrogen saturated glove box. The mixture was stirred for 30 minutes at room temperature and then heated at 120 ℃ for 96 h. After the reaction, the solution was cooled, 40 mL of anhydrous dichloromethane was added and then subjected to vacuum filtration under nitrogen in the glove box. The solid was washed three times with a total of 15 mL of additional dichloromethane to remove dichlorodimethylstannane (5). The rest of the dichlorodimethylstanane was removed by a sublimation technique to obtain pure 3.85 g (9.6 mmol) of Bis-phenanthren-9-yl-chloro-borane (10). Product yield 74.4%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (2H, q, J = 7.48 Hz), 7.65 – 7.73 (2H, m), 7.81 (1H, d, J = 7.70 Hz), 8.09 (1H, s), 8.49 (1H, d, J = 8.11 Hz), 8.74 (1H, d, J = 8.11 Hz), 8.80 (1H, d, J = 8.11 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 122.79, 123.32, 126.73, 126.92, 127.13, 128.07, 128.75, 129.49, 129.62, 130.43, 131.25, 131.82, 134.23, 137.35; ^{11}B NMR (128 MHz, $CDCl_3$): δ 64.63.

2.2.2c Synthetic Procedure of bis-phenanthren-9-yl-(2-methylenecyclopropyl)-borane (14), **BPNMB**

An oven-dried 100 mL Schlenk flask cooled at -78 ℃ was charged with 0.544 g (0.66 mL, 10.1 mmol) of MCP (11) in 15 mL of anhydrous THF. To the solution, 3.9 mL of 2.5M n-BuLi (9.75 mmol) in hexane was added slowly and stirred at room temperature for 2 h. The mixture was cooled at -50 ℃ and 3.85 g (9.6 mmol) of bis-phenanthren-9-yl-chloro-borane (10) dissolved in 20 mL of dry THF was added slowly over 15 min via cannula. The reaction mixture was stirred at room temperature for 24 h. The solution was filtered to remove salt and concentrated in vacuo. The solid was dissolved in 25 mL of anhydrous dichloromethane and filtered with a syringe filter to get a clear solution. The solution was concentrated in vacuo and dried under vacuum to obtain 1.73 g (4.14 mmol) of the final product, **BPNMB** (14). Product yield 43.1%. ^{11}B NMR (128 MHz, $CDCl_3$): δ 48.79.

2.3 GC Analysis

For all GC analysis, a Hewlett-Packard 5890 GC with capillary column (30 m x 0.25 mm i.d.) coated with a 0.25 μm film of 5% phenyl methyl silicon and a flame ionization detector was used. The temperature of oven was programmed at 30 ℃ isothermal with an injection point temperature of 50 ℃. The detector was operated at 235 ℃ and sample was injected under split less condition. Helium was used as carrier gas with a 1.5 mL/min column flow.

*2.4 Sample Preparation of **BNMB** and **BPNMB** for Controlled Release Analysis of 1-MCP*

Two separate GC analyses were conducted to study the controlled release capability of 1-MCP. For **BNMB**, 272 mg (0.85 mmol) of sample and for **BPNMB**, 321 mg (0.77 mmol) of sample, were each mixed with 0.8 mL of H_2O in a 1.5 mL air tight vial at room temperature (22±1 ℃). The head space vapors collected from the solutions were injected into the GC.

*2.5 Comparative Study of Release of 1-MCP from **BPMB**, **BNMB** and **BPNMB** at Different Time Intervals*

Three air tight 4 mL vials were separately charged with equivalent amounts (0.79 mmol) of **BPMB** (291 mg), **BNMB** (254 mg) and **BPNMB** (329 mg) respectively and 0.8 mL of water in each vial. All the samples were prepared at room temperature (22±1 ℃). The mixtures in each vial were vigorously stirred throughout the 99 h experiment. The vapors collected from the head spaces of the vials were injected into a GC to quantify the accumulated 1-MCP for four different time segments, 0-21 h, 22-46 h, 47-76 h and 77-99 h. After each segment the flasks were kept under vacuum for 10 minutes and then flushed with nitrogen.

2.6 Experimental Procedure for the Impact of Environmental Stimuli on the Release Rate of 1-MCP from B-MCP Complexes

Two B-MCP complexes (**DCMB**, **BNMB**) were chosen to analyze the impact of humidity, surface area, water pH and amount of water on the release rate of 1-MCP. Two different humidities (95% and 44%), two surface areas (7.06 cm^2 and 10.75 cm^2), three pH (3.9, 6.8, 10.6) and four different volumes of water (0.1 mL, 0.4 mL, 0.7 mL 1.0 mL) were used for a specific amount of complexes in each type of analysis. All the samples for GC analyses were prepared at room temperature (22±1 ℃).

2.6.1 Impact of Humidity on B-MCP Complexes in Release of 1-MCP

A total of eight 250 mL air tight glass jars with screw caps and rubber septa were used. Four of the jars were loaded with a supersaturated solution of $Na_2HPO_4.12H_2O$ (30 g salt + 30 mL water) and four were loaded with K_2CO_3 (30 g salt + 15 mL water) salts to provide 95% and 44% humidity conditions, respectively, inside the jars. A tripod stand was placed in each jar supporting a petri dish of surface area 10.75 cm^2 (Figure 5). Four of the petri dishes were loaded with 150 mg (0.65 mmol) of **DCMB** and the other four dishes with 100 mg (0.31 mmol) of **BNMB**. The samples (**DCMB**, **BNMB**) were dissolved in the least amount of anhydrous dichloromethane and then equally distributed in the petri dishes to obtain a homogeneous layer of sample. Loaded petri dishes were dried under vacuum before placing them in the humidity chambers. Two petri dishes from each group were then placed in the four jars with 95% humidity and the other two dishes from each group were placed in the four jars with 44% humidity to generate duplicate analyses. GC readings for accumulated 1-MCP were measured from the headspace of the jars at different time intervals.

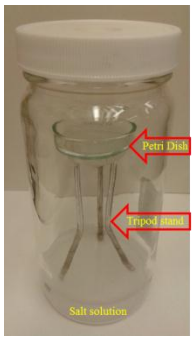

Figure 5. Humidity chamber

2.6.2 Impact of Surface Area on B-MCP Complexes at Specific Humidity (95%)

Eight 95% humidity chambers were designed as mentioned above. Four of them were used for the **DCMB** and the other four chambers were for the **BNMB** complex. Two different sizes of petri dishes (surface area: 7.06 cm^2 and 10.75 cm^2, respectively) were placed in each two **DCMB** chambers containing same amount (150 mg, 0.65 mmol) of **DCMB**. The samples were dissolved in the least amount of anhydrous dichloromethane and then equally distributed in the petri dishes to get a homogeneous layer. Loaded petri dishes were dried under vacuum before placing them in the humidity chambers. Same design of analysis was used for **BNMB** complex containing 100 mg (0.31 mmol) of sample in each petri dish. GC readings for accumulated 1-MCP were measured from the headspace of the jars at different time intervals.

2.6.3 Impact of Water pH on Release Rate of 1-MCP from B-MCP Complexes

Six 2 mL airtight vials were charged with 56 mg (0.24 mmol) of **DCMB** individually where, each two vials were added 0.4 mL water of pH 3.9, 6.8 and 10.6 respectively. Quantification of the accumulated 1-MCP from the head space of the vials at the respective pH were made by GC analyses at different time intervals. Between the time intervals the mixtures were continuously stirred. The same procedure was followed in the case of **BNMB** analysis, charging each vial with 51.8 mg (0.16 mmol) of sample.

2.6.4 Effect of Water Volume on the Rate of Hydrolysis Reactions to Release 1-MCP

Eight 2 mL air tight vials with screw caps and septa were charged with 57.2 mg (0.25 mmol) of **DCMB** in which, 0.1, 0.4, 0.7 or 1.0 mL of water, respectively, was added in two of the vials with continuous stirring. The released 1-MCP was collected from the head space of each vial and quantified with GC analysis at specific intervals. After each GC injection, the vials were opened and flushed with nitrogen flow to make sure there was no 1-MCP left in the vials which was released during the previous intervals. For the analysis with **BNMB**, the same procedure was followed charging each vial with 52.2 mg (0.16 mmol) of sample.

2.7 Contact angle Measurements for Hydrophilicity/Hydrophobicity Characteristics

The contact angle (θ) is the angle formed between the surface of a liquid and the outline of the contact surface to a solid where a liquid-vapor interface meets a solid surface at a specific temperature and pressure and quantifies the wettability of a solid by a liquid by Young's equation (Kwok & Neumann, 1999). In this study with water as the wetting liquid, the solid surface can be classified as hydrophilic or hydrophobic (Förch, Schönherr & Jenkins, 2009). If a water drop spreads out over a surface of a solid, the attraction between the solid and liquid is strong and the contact angle is 0 °. For contact angles between 0^o and 90^o, the solid is considered as hydrophilic and above 90^o it is considered as hydrophobic.

To measure the contact angle of water droplets on the B-MCP complexes, glass slides were prepared with a thin homogenous layer of each B-MCP complex (**BPMB**, **BNMB** and **BPNMB**). The thin films of B-MCP complexes were made by placing 10 mg of each complex dissolved in 0.5 mL of dichloromethane on individual slide. A contact angle Automated Goniometer (Ramé-hart Instrument Co., Succasunna, NJ, USA) equipped with a high resolution camera (Model 590 F4 Series) was used to determine the hydrophilicity and wettability of the complexes. Experiments were carried out in air through static sessile drop measurements using ultrapure water as the liquid phase (11 ± 0.6 μL drops). The distance between needle-tip and compound surface was kept constant to ensure consistency between the different measurements. The reported angles were recorded after ≤10 s to avoid as much as possible penetration of water into the surface layer of the complexes. Determination of the contact angles of the droplets were performed using Dropimage Advanced v2.5 software. The results were the average of five to ten water drops deposited on each compound layer surface.

3. Results and Discussion

There is no existing technology available for use of 1-MCP in an open environment as it is a gas at ambient conditions, although it has been shown that 1-MCP can potentially protect crops from environmental stresses such as drought to reduce yield loss (Kawakami & Oosterhuis, 2006; Kawakami et al., 2010). Our invented B-MCP complexes (Figure 2) have shown enormous potential for open field applications as they are stable under ambient conditions owing to higher boiling points than 1-MCP and capable of releasing 1-MCP for a long time period from hours to days depending on their structural differences and availability of moisture. In our previous article (Sarker et al., 2016) the effectiveness of two B-MCP complexes (**DCMB**, **BPMB**) in an open environment was reported in which they were found active in delaying the ripening process of matured green tomatoes for more than 7 days. These encouraging results obtained in the laboratory have driven us to develop new B-MCP complexes and to check their effectiveness under environmental stimuli such as humidity, water pH, water volume and surface area. In synthesizing new B-MCP complexes, polycyclic aromatic hydrocarbons (PAH) were chosen to provide the branch chains of the complexes (Figure 3, **BNMB** and **BPNMB**) as they are readily available, cheap, possess pesticidal properties and are biodegradable. A four-step synthetic scheme (Figure 4) for **BNMB** (13) and **BPNMB** (14) resulted in good yields at every step. An important part of this synthetic route is the recovery of dichlorodimethylstannane (5) in the following step that can be reused thus reducing the synthetic costs.

The B-MCP complexes with PAH (**BNMB**, **BPNMB**) as side chains could be used for dual purposes such as in controlled release of 1-MCP and as a pesticide for crop fields. For example, after complete release of 1-MCP from **BNMB** and **BPNMB**, naphthalene and phenanthrene, which are well known pesticides, will be deposited, respectively, due to uncontrolled and complete hydrolysis of B-MCP complexes. Naphthalene has been used as a household fumigant. Other fumigant uses of naphthalene include use in soil as a fumigant pesticide. Naphthalene is broken down by soil bacteria to naphthalene diol, salicylic acid, and catechol. Some bacteria may utilize naphthalene as their sole carbon source (Cerniglia, 1984). Bacterial oxidation pathways include five metabolites of naphthalene along the degradation pathway: cis-1,2-dihydroxy-1,2-dihydronaphthalene, 1,2-dihydroxynaphthalene, cis-o-hydroxybenzalpyruvic acid, salicylic acid, and catechol, which was subsequently subject to ring cleavage (Bouwer, J., McCarty, Bouwer, H. & Rice, 1984). Fungal degradation of naphthalene produces naphthalene-1,2-oxide via cytochrome P-450 oxidation. The oxide can be subsequently hydrolyzed to trans-1,2-dihydroxy-1,2-dihydronaphthalene, or alternatively conjugate with glucuronide or sulfate to break down first to 1-naphthol and 2-naphthol and subsequently to 4-hydroxy-1-tetralone (Jury, Spencer & Farmer, 1984). Other reported metabolites include naphthalene trans-1,2-dihydrodiol, 1,2-naphthoquinone, and 1,4-naphthoquinone (Garon, Krivobok, Seigle-Murandi, 2000). Phenanthrene could be an alternative option as it is used as pesticides and also biodegradable (Shetty et al., 2015).

*3.1 Controlled Release of 1-MCP from **BNMB** (13) and **BPNMB** (14)*

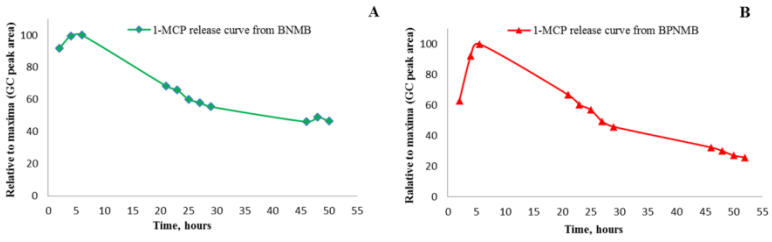

Figure 6. Controlled release of 1-MCP from **BNMB** and **BPNMB** when in contact with water

As shown in Figure 6, **BNMB** (13) and **BPNMB** (14) are capable of gradual release of 1-MCP when in contact with water. Both **BNMB** and **BPNMB** require close to 6 h to reach their highest point of release and after that both descending curves indicate either completion of 1-MCP release from the complexes or much slower release rate accumulating less amount than the amount of 1-MCP required to be pulled out from the airtight vials to obtain an ascending curve in GC analysis. The curves (Figure 6) also show **BNMB** and **BPNMB** are more reactive toward water than **DCMB** (Figure 2a) (Sarker et al., 2015) in which the highest point was reached in 21 h. The faster release of 1-MCP makes **BNMB** and **BPNMB** better choices for applications which require relatively high concentrations of 1-MCP for a short period of time. The differences in release rate of 1-MCP from the B-MCP analog is controlled by the mechanism of the hydrolysis reaction (Figure 7). The driving force of these particular hydrolysis reactions are generated differently due to the structural differences of the attached moieties of the B-MCP complexes. Following the mechanism (Figure 7), it is unquestionable that the more

electron deficient the central boron atom is, the faster the nucleophilic attack by the hydroxyl group of the water molecule will be, leading to the cleavage of the carbon-boron bond to produce 1-MCP. In this regard, the central boron atom in **DCMB** is more electron rich than that in **BNMB** or **BPNMB** because of two electron releasing cyclohexyl groups present in the DCMB structure. Alkyl groups are known to be electron donating groups because of inductive effects and also hyperconjugation. As a result of being electron rich, the boron atom in **DCMB** is more resistant toward nucleophilic attack than in **BNMB** or **BPNMB** offering better control in release of 1-MCP.

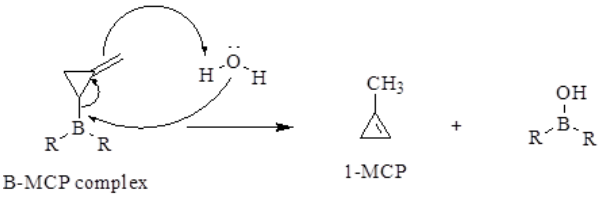

Figure 7. 1-MCP releasing mechanism from B-MCP complexes; R= alkyl or aryl group

*3.2 Comparative Study of 1-MCP release from **BPMB**, **BNMB** and **BPNMB***

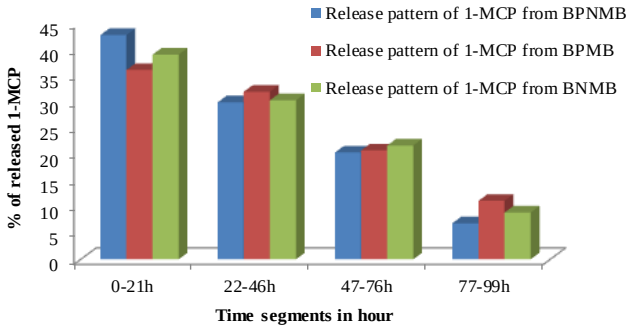

Figure 8. Comparative release pattern of 1-MCP from **BPNMB**, **BPMB** and **BNMB** when in contact with water

From this comparative study in Figure 8, it is shown that **BPNMB**, **BNMB** and **BPMB** release 1-MCP at a similar rate when in contact with water. In first 21 h, they release 42%, 39% and 36% of total released 1-MCP in 99 h, respectively. These slight differences in releasing 1-MCP in the first 21 h could be resulted from the differences in their resonance stabilities. The B-MCP complexes **BPMB**, **BNMB** and **BPNMB** have polycyclic aromatic hydrocarbons (PAH) in their structures in which internal resonance is possible (Figure 9) making these molecules stable and at the same time, the central boron atom becomes electron deficient due to the delocalization of π-electrons and thus vulnerable to nucleophilic attack by the hydroxyl groups of H_2O. The attached moieties of **BPNMB** (phenanthrenyl) have the highest number of cyclic aromatic rings creating stronger internal resonance than in **BNMB** and **BPMB** and thus make the central boron atom the most electron deficient ($B^{+\delta}$), which favors faster hydrolysis. The branches of **BNMB** (naphthalenyl) and **BPMB** (biphenyl) have the same number of cyclic aromatic rings but in the case of **BPMB**, the phenyl rings connected with a single σ-bond inhibit to provide the full impact of resonance on the central boron atom than in **BNMB** which permits hydroxyl group interaction as a slower attack on **BPMB**.

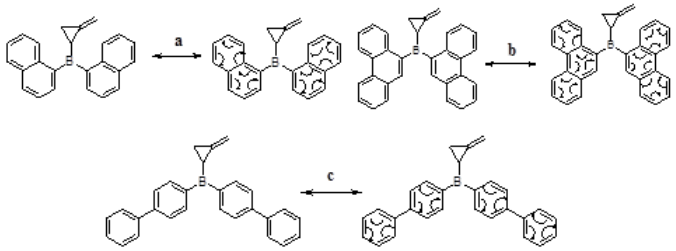

Figure 9. Resonance structures of a) **BNMB**, b) **BPNMB** and c) **BPMB**

The differences in the release rate of 1-MCP from B-MCP complexes may also be rationalized by their hydrophobic and hydrophilic characteristics. Hydrophobicity and hydrophilicity of a compound were determined by contact angle measurement analysis. The contact angles of **BPMB**, **BNMB** and **BPNMB** with a water droplet

were measured as 99.5 ± 3°, 76.5 ± 3.5° and 67.0 ± 2°, respectively, suggesting that **BPMB** has hydrophobic character whereas **BNMB** and **BPNMB** have hydrophilic characteristics. **BNMB** and **BPNMB** with hydrophilic character attract water molecules more strongly than **BPMB** leading to a faster hydrolysis reaction. Similarly, **BPNMB** is more hydrophilic than **BNMB** allowing increased contact with the water molecules and a faster hydrolysis reaction. It is also important to mention that all three B-MCP complexes are capable of releasing 1-MCP even after 77 hours with at least 5% or more released. This variation in release rate will help to select the right complex for specific applications (Figure 8).

3.3 Effect of Environmental Stimuli on Releasing 1-MCP from B-MCP Complexes

3.3.1 Effect of Humidity

DCMB is found to release 1-MCP at a faster rate at 95% humidity than at 44% humidity as expected (Figure 10A). After two days, **DCMB** released 22% of its maxima at 95% humidity whereas only 6% of the maxima was released at 44% humidity. Although the maximum release rate of 1-MCP for both humidities were reached at 16 days, in the case of 95% humidity, the release rate was faster than at lower humidity on its way to maxima. It might have something more to do with the dew point. At 44% humidity and 22 °C and in atmospheric pressure, the dew point is about 9 °C. This means that there is little water to trigger 1-MCP release. At 95% and 22 °C and in atmospheric pressure, the dew point is about 21 °C, and there is more moisture causing release of the 1-MCP. On the other hand, **BNMB** (Figure 10B) did not show any significant difference in release rate of 1-MCP between 95% and 44 % humidity conditions because of its high reactivity toward water. It was found to reach 80% of the maxima in one day and maximum in three days in both humid conditions. Therefore, the releasing capability of 1-MCP under humidity will make these B-MCP complexes useful in crop field without rain or water sources.

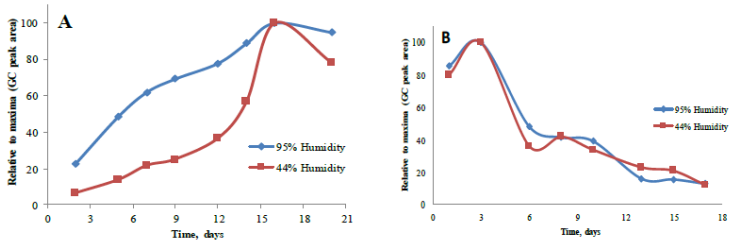

Figure 10. The effect of humidity at 22±1 ℃ on release rate of 1-MCP from A) **DCMB** and B) **BNMB**

3.3.2 Effect of Surface Area

At 95% humidity, **DCMB** was found to release 1-MCP at a faster rate with the larger surface area (Figure 11a). In both cases 20% of the maxima was released in the first two days and the maxima was reached at 16 days. Again in the case of **BNMB** (Figure 11b), no significant differences were observed in release rate of 1-MCP from two different surface areas due to its high reactivity. Release rate of 1-MCP can be controlled by changing the exposed area of B-MCP complexes which will provide an additional tool to manipulate this technology in open field applications.

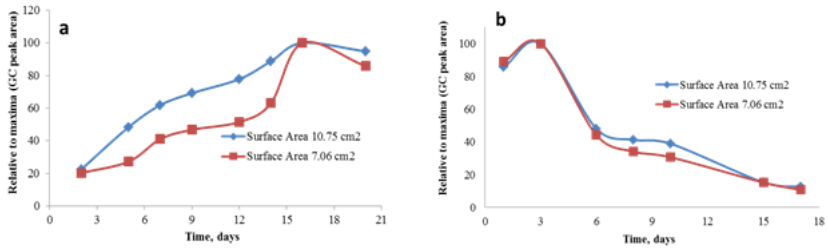

Figure 11. Effect of Surface Area on release rate of 1-MCP from a) **DCMB** b) **BNMB** at 95% humidity.

3.3.3 Impact of Water pH

In the case of **DCMB**, the release rate of 1-MCP was found to vary with the pH of water (Figure 12a). At basic conditions (pH 10.6), the rate of hydrolysis was increased resulting in a faster release rate of 1-MCP than at pH

6.8 or 3.9. Although it took about 27 h to reach the maxima at pH 6.8 or 10.6, the sudden drop of the release curve for pH 10.6 indicates the fastest rate as it released most of the 1-MCP within that time. On the other hand, at pH 3.9, the maximum was reached in 74 h. However, there was no significant difference observed in the release of 1-MCP from **BNMB** (Figure 12b) at pH 3.9 or 6.8, but similar to **DCMB** at pH 10.6, the release rate became faster by releasing the most part of 1-MCP on its way to reach the maximum point of 28 h. The independency on release rate of 1-MCP from B-MCP complexes by water pH will expand their application in different geographical locations with pH differences in rain water.

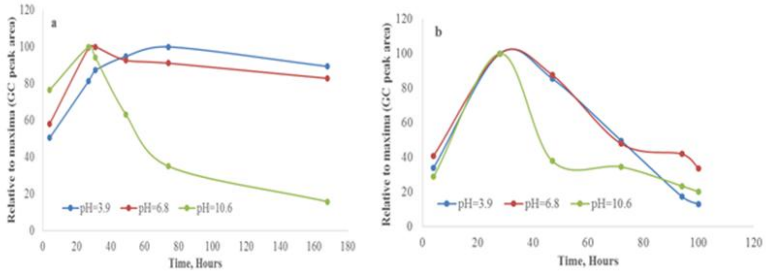

Figure 12. Effect of water pH on release rate of 1-MCP from a) **DCMB** b) **BNMB**

3.3.4 Effect of Water Volume

From Figure 13a, it is clear that, the reaction rate of hydrolysis of **DCMB** is changed up to the sufficient amount of water (0.1 mL) and after that the rate becomes independent to more water content. That is why 63% of total 1-MCP was released in first 48 hours in 0.1 mL of water where 76 % (±2) remained unchanged in 0.4-1.0 mL of water. In case of **BNMB**, very little or no change in 1-MCP release were observed due to the amount of water (Figure 13b) which is consistent with the humidity experiments where **BNMB** was found to release 1-MCP at similar rates under two humidity conditions (95% and 44%).

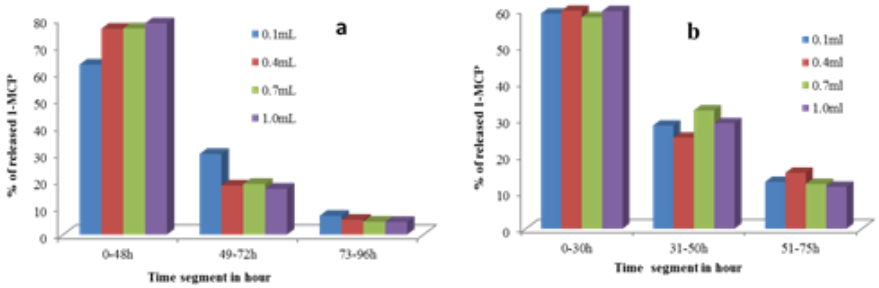

Figure 13. Effect of water volume on release rate of 1-MCP from a) **DCMB** b) **BNMB**

4. Conclusion

Two new B-MCP complexes have been synthesized which are capable of releasing 1-MCP for up to 99 h when in contact with a sufficient amount of water. It was found that they release 1-MCP at a faster rate in the beginning than the previously reported complexes, **DCMB** and **BPMB**. This difference in release rate is due to either electronic effects resulting from the internal resonance of the PAH groups or the hydrophilic characteristics of the B-MCP complexes or both. It is proven that B-MCP complexes are also capable of releasing 1-MCP not only in contact with water but also under humidity. The other factors like surface area, water pH which are of importance when considering use in open field applications. The change in environmental conditions impacts the release rate of 1-MCP from B-MCP complexes unless they are too sensitive to water molecules (hydrolysis). Overall, these B-MCP analog complexes have shown enormous potential as a means to provide 1-MCP in open environments over a long period to protect crops in hostile environmental conditions. The control of 1-MCP release from B-MCP complexes by water pH is an important finding to utilize this technology.

Acknowledgment

We thank Dr. Gary Strahan for his help with acquiring and processing the data for NMR analyses of the

compounds. We also thank Mr. Raymond Kwoczak for his assistance in acquiring the contact angles of the B-MCP complexes.

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