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CHEMICAL BASIS FOR REPELLENCY OF *Sargassum cinctum* J. AGARDH (Sargaceae) AGAINST ASIAN CORN BORER, *Ostrinia furnacalis* (Guenee) (Lepidoptera:Crambidae)

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ABSTRACT

Anecdotal claims reveal that Sargassum seaweed has long been used by farmers to reduce insect pest populations in cacao and various vegetables. A study was conducted at the National Crop Protection Center, College of Agriculture and Food Science, University of the Philippines Los Baños from 2015-2017 to validate farmer knowledge on reduced Asian corn borer populations when brown seaweed, *Sargassum cinctum* is placed in plot borders. Prominent volatile organic chemicals (VOCs) emitted by seaweed under sunlight and room conditions were identified by GCMS and differences in emission patterns were observed. In general, the fresh seaweed extract showed a higher percent repellency as compared to the dried seaweed extract. We identified for the first time the nature of the compounds emitted by *S. cinctum* and repelling the Asian corn borer. Some VOCs presented in combination did not produced synergistic effect. The number of eggmasses and number of eggs laid by female moths was reduced when brown seaweed was applied on corn under field cage conditions. Sargassum seaweed can be used together with other effective IPM strategies, especially in coastal areas, to reduce corn borer population. Brown seaweed can be an effective pest management strategy tool for low input and/or organic green corn production

Key words: insect behavior, semiochemicals, allomone, pest management

INTRODUCTION

Anecdotal claims indicate that brown seaweed, *Sargassum cinctum* has long been used by farmers to reduce insect pest populations in cacao and various vegetables. Preliminary trials in corn, conducted in barangay Poblacion, San Andres, Quezon in Bondoc Peninsula from June to September 2013 by Felipe Duaso, a farmer-scientist trained under the Farmer Scientist Training Program (FSTP) of the University of the Philippines, demonstrated higher yields in the plots with seaweed at the edges of the plot, compared to control plots. He claims this is farmer-knowledge gained from the experiences of his father and other farmers in their farming community. This observed phenomenon is similar to the larvicidal activity of certain seaweeds and algae against mosquitoes (Hira et al. 2010; Thangam and Kathiresan 1996).

The production of volatile organic compounds (VOC) by plants is essential for chemical ecology interactions. Headspace collection of volatiles have demonstrated the presence of major volatile compounds found in seaweeds: hydrocarbons, terpenes, phenols, alcohols, aldehydes, ketones, esters, fatty acids and halogen or sulfur-containing compounds (Gresslera et al. 2009). Non-host plant volatiles interfere with orientation to the host plant and affect the olfactory, feeding and oviposition behavior of major insect pests and even natural enemies (Brevault and Quilici 2010; Bruce and Picket 2011; Finch and Collier 2000; Forbes and Feder 2006; Koschier et al. 2000; van Toll and Visser 2002). Biologically active plant volatiles may be used in the development of integrated pest management strategies (Koschier et al. 2000; Calumpang et al. 2013 and 2014).

Filipino farmer knowledge on insect pest reduction when tagbak (*Alpinia elegans* (C. Presl) K. Schum) stalks are placed in rice fields also served to reduce insecticide use in rice production in Infanta, Quezon. Volatile organic chemicals emitted by tagbak such as α-terpinene, α-pinene, and cymene demonstrated 67 to 70% repellency against the green leaf hopper, a vector of tungro in rice. Therefore, the use of tagbak for insect pest management in rice production can be promoted in areas where these abound, to reduce dependence on synthetic insecticides (Calumpang et al. 2012; Calumpang et al. 2013).

Kakawate (*Glyricidia sepium*) (Jacq.) Walp., is also believed to repel insect pests of rice, while leaf bunches are placed close to lighted bulbs in night fairs and markets to repel insects. Volatile chemical components of kakawate were demonstrated to repel green leaf hopper, *Nephottetix virescens* in olfactometric bioassays. Volatile chemicals emitted by kakawate were identified by GC/MS (Calumpang et al. 2013). Both plants are used by organic rice farmers in the Philippines (Pantoja et al. 2016).

Asian corn borer is a major pest of corn, damage has been found to result in tremendous yield reduction ranging from 20 to 80% (Sanchez 1971). It comes as a result of infestation from two generations during a cropping season. The main point of establishment and survival of the 1st and 2nd instars of the first generation are on the whorled leaves while the 3rd and 4th generation prefer the leaf sheaths (Magsino 1995). The Asian corn borer is repelled by a plant volatile, 1-methylethyl propyl disulfide, which is emitted by both corn and a weed closely associated with corn, *Ipomoea triloba*. The emission is significantly increased when corn is entwined by the weed (Calumpang et al. 2000). This study provided the chemical basis for the population reduction of corn borer in corn fields with *I. triloba*.

Other cultural management practices should therefore be tested to make available additional alternatives to chemical pesticides. The increase in yield would redound to less inputs and lower risk for farmer applicators, consumers and the environment. The potential impact of this research, includes among others: reduced insecticide use in corn production, additional pest management options for organic agriculture and less environmental and human health concerns regarding pesticide use in corn production.

In the present paper, our objective was to validate farmer knowledge on reduced insect pest populations in corn with fresh brown seaweed.

MATERIALS AND METHODS

Establishment of stock culture

The parental stock for the mass rearing of the Asian corn borer (ACB) was collected from Los Baños, Laguna, Philippines. The larvae were reared at the National Crop Protection Center (NCPC), College of Agriculture, UPLB. An artificial diet was used to feed the larvae which were 1-2 weeks old and were placed in plastic rearing cups. On the third week, the larvae were placed in a large container until they pupate and were fed with young corn and its stalks. The pupae were collected daily and transferred to a separate container. The newly-emerged adults were fed with a sugar solution-soaked cotton hung at the top of the container. Also, egg masses collected in the cage bioassays served as an additional source ACB for rearing/stock culture.

Corn planting

Six corn seeds were sown in pots and were thinned to 3 plants per pot after 2 weeks. The corn plants were then fertilized with complete fertilizer (14-14-14) at the rate of 15g per 4 li water, every 2 weeks and watered daily. Corn planting was done weekly in order to ensure staggered growth of the plants.

Seaweed

The seaweed samples used in various experiments were collected from Catanauan, Quezon Province, Philippines in 2015 and from Calatagan, Batangas in 2016. These were used as fresh material or air dried. The seaweed was identified as brown seaweed, *Sargassum cinctum* J. Agardh, (Fig. 1) by Dr. Garmino C. Trono, Professor Emeritus, Institute of Marine Science, University of the Philippines Diliman, Quezon City, Philippines.

Fig. 1. Immature and mature brown seaweed, *Sargassum cinctum*.

Extraction of headspace volatiles emitted by Sargassum seaweeds

The volatile organic chemicals (VOCs) emitted by *Sargassum* seaweed samples were collected by enclosing the sample in a Tygon bag and subjecting it to Tenax adsorption, followed by Soxhlet extraction (42 °C) in *n*-pentane and concentrated in a micro Kuderna Danish set-up (34 °C). The final extracts were diluted in *n*-hexane and were kept frozen until analysis by GCMS.

The volatile components were analyzed using a Shimadzu 2010 GCMS at Natural Science Research Institute (NSRI), University of the Philippines Diliman. The temperature settings of the capillary gas chromatography-mass spectrometer was maintained at 60 °C for 3 min, and programmed at 5°C /min to 250 °C and held for 5 min. Injector temperature was maintained at 250 °C. Compound identification was based on mass spectra analysis completed with the mass spectral database NIST107.Lib.

Olfactometric bioassays

Seaweed extracts (0.5 mL) were pipetted to a filter paper and were allowed to dry. Treated and control (hexane-treated) filter papers were inserted at opposite ends of the Y-tube olfactometer. Adult female Asian corn borers were placed at the open end of the olfactometer and vacuum was applied. It was then covered with carbon paper and was observed for 10-15 minutes. One ACB per replicate was done, and a total of 30 replicates were conducted.

Individual reference materials of volatile organic compounds (Sigma, USA), identified by GC-MS, were assayed for repellency against corn borer females.

Effect of *S. cinctum* on oviposition of ACB in field cage experiments

One hundred twenty (120) corn plants per set-up were used (6 rows with 20 corn plants each) for the field plot bioassay. One set-up contained the corn plants with seaweeds, while the other set-up contained corn plants only. A two meter distance between the two treatments was provided. The set-up were enclosed in a muslin cloth cage, properly fastened on the sides to ensure that no insect can escape. Twenty five (25) pairs of newly emerged Asian corn borers were released at the center of the cage to allow ovipositing females to select oviposition sites.

Statistical analysis

The data obtained in the field cage experiments were analyzed statistically and significant differences of means were determined using Independent Sample T-test (SAS Institute 2001).

RESULTS AND DISCUSSION

Identification of headspace volatiles emitted by *Sargassum cinctum*

The compounds present as volatile organic chemicals (VOC) emitted by seaweed were elucidated through GC-MS analysis. Tentative identification showed the prominent chemical components are: 2-ethyl-1-hexanol, 2,2,4-trimethylheptane, 1,3,5-undecatriene, 6-[(Z)-1-butenyl]-4-cycloheptadiene, 2,4-di-tert-butylphenol and 1-butoxy-2-ethylhexane. The minor components are 3-eicosene, 9-eicosene, 3-butyl-4-vinylcyclopentene, 3-(1-butenyl)-4-vinyl-cyclopentene, 6-butylcyclo-1,4-heptadiene, and dipropyl disulphide..

Prominent VOCs of mature *S. cinctum* were: 1,3,5-undecatriene and 6-(1-butenyl)cyclohepta-1,4-diene (Fig. 2). These were consistently emitted by Sargassum samples for 3 successive days.

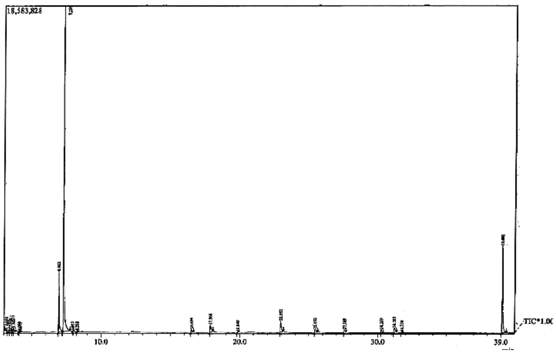

Fig. 2. Representative gas chromatography-mass spectrometry trace of headspace volatiles released from mature *Sargassum cinctum*

Volatile organic chemicals emitted by immature *S. cinctum*were slightly from the emission profile of mature seaweed. Prominent VOCs in immature *S. cinctum* were: 2-ethyl-1-hexanol, 6-[(Z)-1-butenyl]-1,4-cycloheptadiene and 1,3,5-undecatriene (Fig. 3).

6-[(Z)-1-butenyl]-1,4-cycloheptadiene is reported to be present in brown alga *Dictyopteris membranacea,* (Hattab et al. 2007) and (*Ectocarpus siliculosus*) as algal sexual pheromone (Tringali 1997) while 1,3,5-undecatriene was part of the volatile fractions of brown alga, *D. membranacea,* along with 3-butyl-4-vinylcyclopentene and 6-butylcyclo-1,4-heptadiene (Hattab et al. 2007). Minor components include: 5-methyl-3-heptanone, 3,5,5-trimethyl-1-hexene, 2-methyl-4-heptanone, 12-methyltetradecanoic acid methyl ester which is reported to be present in green alga (*Spirogyra longata*), possibly derived from fatty acids (Abdel-Aal et al. 2015).

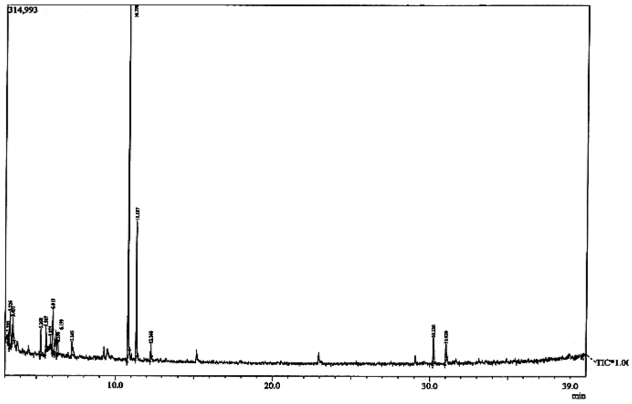

Fig. 3. Representative gas chromatography-mass spectrometry trace of headspace volatiles released from immature *S. cinctum*.

The production of VOC is closely related to the physiology of the species (Gresslera et al. 2009). Different growth conditions of the same species are implied by differences in VOC profiles (Kajiwara et al. 1989).

Several volatile compounds emitted by *S. cinctum* have been determined from other brown algae and *Sargassum* species. The 3-(1-butenyl)-4-vinyl-cyclopentene, 6-(1-butenyl)cyclohepta-1,4-diene and 1,3,5-undecatriene are known algal sexual pheromones of brown marine alga (Tringali 1997; Maarse 1991; Boland et al. 1983; Hattab et al. 2007). 2-Ethyl-1-hexanol has also been identified in *Sargassum subrepandum* (Forsk) (Abou-El-Wafa et al. 2011).

Full sunlight conditions however, resulted in an entirely different emission pattern. VOC emissions under full sunlight showed a different pattern from the emissions made under room conditions (24°C, ambient room light). Prominent emissions at higher temperatures (30 – 35°C) under full sunlight were 2-ethylhexylglycidyl ether and 2,4-di-tert-butylphenol (Fig. 4). These were not detected in samples collected under room temperature conditions. Minor compounds include: 2,3,8 trimethyldecane, eicosene, and 4-heptadecenal. The emission of volatile alcohols was noted, namely, 1-dodecanol, and heptanol.

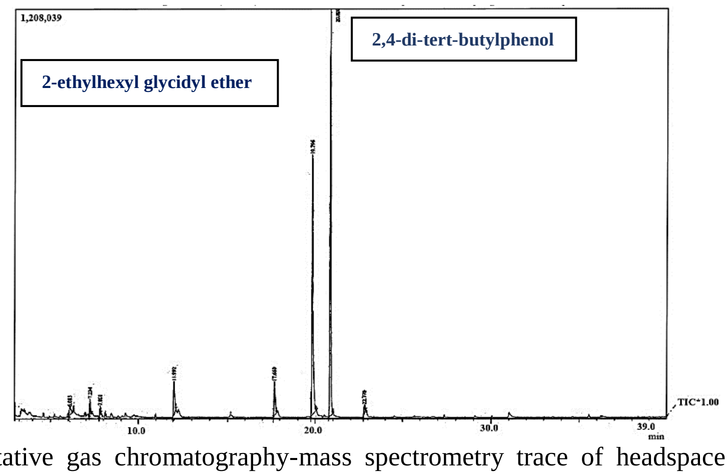

Fig. 4. Representative gas chromatography-mass spectrometry trace of headspace volatile profile released from *Sargassum cinctum* under full sunlight (30 – 35°C).

Eicosene has been reported in *Sargassum wightii* (Pandiyan et al. 2014). The presence of 2,4-di-tert-butylphenol has likewise been reported in four red algae: *Gracilaria bursa-pastoris, Phyllophora crispa*, *Laurencia obtusa* var. pyramidata and *Jania rubens* (Guven et al. 2014; Karabay-Yavasoglu et al. 2007) as well as in steam distillate of *Dictyopteris membranaceae and Cystoseira barbata* (Ozdemir et al. 2006).

Volatile emissions under full sunlight is expected to be different from volatile emissions under room temperature. It has been established that temperature has a strong effect on the quantity of volatiles released in the headspace. Emission at 10°C was significantly lower than at 15°C and 20°C. This difference can be attributed to a temperature effect on the secretion of volatiles rather than on the evaporation rate of volatiles. Light influenced fragrance emission significantly, the most intense emission being noted at high irradiances (Jakobsen and Olsen 1994).

Water stress is also another factor that affects release of VOCs and explains the difference in repellency produced by fresh and dried Sargassum.

In maize, headspace volatile compounds emitted by watered and water-deprived maize plants had different profiles. The most abundant compounds from watered plants are limonene, linalool, benzoic acid, indole, β-caryophyllene and acetophenone, whereas, in water-deprived plants, limonene, acetophenone, hexanoic acid, benzoic acid and indole are dominant. In addition, (E)-4,8-dimethyl-1,3,7-nonatriene, 6-methyl-5-hepten-2-one, anisole and 1-carvone are undetected in the water-deprived plants (Sole et al. 2010).

The compound, dipropyl disulfide present in the Sargassum extract has a similar structure as dimethyl disulfide, a known volatile compound from marine algae (Moore 1976).

Olfactometric bioassays

Olfactometric bioassays demonstrated the repellency of some *S. cinctum* volatile emissions against the ACB. The Y-tube used in the assays was initially established to be effective in assessing repellency using phenylacetaldehyde, a known attractant of ACB (Cantelo and Jacobson 1979). Phenylacetaldehyde produced 30% repellency in ACB or 70% attractancy (Table 1).

In general, the fresh seaweeds extract showed a higher percent repellency (76.7%) compared to the dried seaweeds extract (61.3%). A lower repellency of the dried seaweeds extract may be due to losses as the chemicals are volatile in nature. A lower concentration of the repellent chemicals renders a lower percent repellency. Thus, fresh brown seaweeds were used in the field trials, as a validation trial of Filipino farmer practice.

Among the VOCs tested, 2-ethyl-1-hexanol, 1-eicosene and 2-ethylhexyl glycidyl ether were the most repellent of the seaweed volatile organic chemicals at concentration levels that approximated the natural ratio. Although 2-4-ditertbutyl phenol is a prominent volatile chemical emitted under sunlight, it did not demonstrate repellency against corn borer moths (Table 1). A mixture of 2-ethyl-1-hexanol and 1-eicosene, both highly repellent (>70% repellency), however did not produce synergistic effect. 2-Ethyl-1-hexanol and dodecanol were reported to be found in *Sargassum subrepandum.* (Forsk) (Abou-El-Wafa et al. 2011) and red alga, *Corrallina elongata* (Dembitsky and Srebnik 2002), respectively. There are no reports of these seaweed species being used for pest management.

Volatile organic chemical emissions play a crucial role in plant-insect interaction. Experiments in which insects have been exposed to plant volatiles alone and in combination have revealed that stronger behavioral responses are obtained with appropriate blends or combinations of volatiles than with single compounds (Bruce and Pickett 2011). 2-Ethyl-1-hexanol was demonstrated to be a part of a six-component blend that was attractive to orange wheat blossom midge, *Sitodiplosis mosellana*

(Diptera: Cecidomyiidae) in an olfactometer bioassay but individual compounds were not attractive at the same dose (Birkett et al. 2004). It was also detected from aeration samples of tea shoots using GC-MS (Mu et al. 2012).

Table 1. Olfactory response of Asian corn borer female moths to volatile organic chemicals reference standards identified in brown seaweed, *S. cinctum.*

Volatile Organic Chemical	Concentration (ug/ml)	% Repellency
2-ethyl-1-hexanol*	30	70
1-dodecanol	0.77	47
1-tetradecanol	0.77	57
1-undecanol	5	57
1-octadecene	5	50
1-eicosene	15	77
2-ethylhexyl glycidyl ether**	5	67
5-methyl-3-heptanone	99.75	37
2-4-ditertbutyl phenol**	60	33
2-ethyl-1-hexanol + 1-eicosene	30 + 15	70
Wet *S. cinctum*	-	77
Dry *S. cinctum*	-	61
Phenylacetaldehyde	15	31

Major peaks: * Room temperature ** Full sunlight

Damaged maize leaves attacked by Asian corn borer larvae release herbivore-induced volatiles that affect orientation behaviors and oviposition of the females. Nineteen volatile chemicals, with terpenes being the major components, were identified. Females responded to (E)-2-hexenal, nonanal, (Z)-3-hexen-1-ol and three unknown compounds while the male only responded to (E)-2-hexenal, nonanal and one unknown compound (Huang et al. 2009).

On a tri-trophic level, studies have demonstrated that herbivore injured plants produce specific blends of odors which can attract certain insect predators and parasitoids (Dicke 1994; Turlings et al. 1995). Herbivore cues are not very detectable to carnivores at a distance, herbivore-induced plant volatiles increase herbivore detectability enormously. Among the terpenoids, the two homoterpenes 4,8-dimethyl-1,3(E),7-nonatriene and 4,8,12-trimethyl-1,3(E),7(E),11-tridecatetraene are the most often reported herbivore-induced plant volatiles. These can be synthesized by plants of many species from the terpene alcohols nerolidol and geranyllinalool without any mediation of herbivory (Dicke 1994).

Females of the braconid larval parasitoid, *Cotesia marginiventris,* are strongly attracted to volatiles emitted by the caterpillar damaged plants. Volatiles emitted by corn seedlings just after caterpillars start feeding on them, include: (Z)-3-hexenal; (E)-2-hexenal, (Z)-3-hexenol, (Z)-3-hexen-l-yl acetate, linalool, (3E)-4,8-dimethyl-1,3,7-nonatriene, indole, a-trans-bergamotene, (E)-,3-farnesene, (E)-nerolidol and (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (Turlings et al. 1995). None of these chemicals were detected in the brown seaweed VOCs. Future work should cover the effects of brown seaweed on the natural enemies and beneficial arthropods in a corn agro-ecosystem.

Effect of *S. cinctum* on oviposition and hatchability of eggs of ACB laid in field cage

There are fewer number of egg masses or eggs laid by adult female ACBs in corn with brown seaweed at 45 days after planting (DAP), both in 2016 and 2017 cropping periods (Fig. 5A and B).

Likewise, hatchability of eggs was greater in corn without seaweed. These indicate that the female corn borer was less attracted for oviposition to corn with brown seaweed and eggs exposed to its volatiles may have weakened the developing embryo resulting to lower hatchability. Air, particularly oxygen is vital for metabolic processes within the egg and the chorion of the egg is permeable to gases. A continuous film of air is held between vertical columns in the inner part of the chorion and the air in the trachea can be kept saturated (Hinton 1960; Hinton 1969). Since VOCs mix with ordinary air, these could penetrate the chorion and possibly exert deleterious effect to the developing embryo. The exact mechanism is still unclear. These results confirmed our earlier laboratory results that fecundity of female ACB and hatchability of eggs was affected by volatiles emitted by the brown seaweed both in rearing pan and Petri plate bioassays (Navasero et al. 2016).

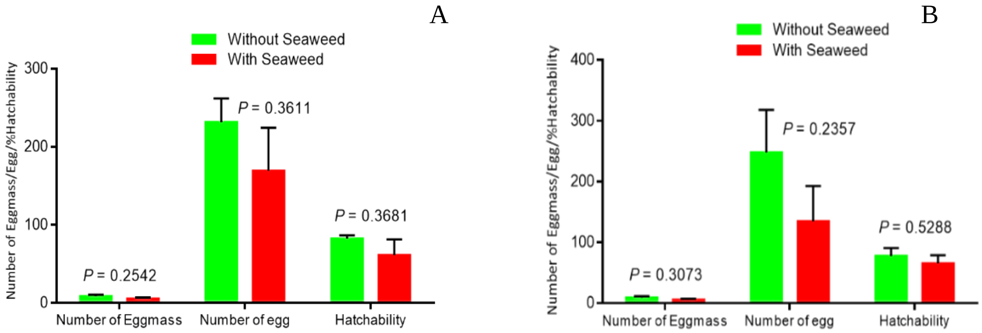

Fig. 5. Mean (±SE) number of egg mass , total number of eggs and percent hatchability of eggs in *O. furnacalis* released in field cage with 45 DAP corn with and without seaweed, *S. cinctum* during 2016 (A) and 2017 (B) cropping period.(t-test; p-value<0.05 was considered significant).

CONCLUSIONS

Filipino farmer innovation in using brown seaweed for corn pest management has scientific basis. Female corn borer moths were less attracted for oviposition on corn with brown seaweed. A reduction in number of egg masses and number of eggs was observed when seaweed was placed on the corn plant. Olfactometric assays demonstrated the presence of volatile organic compounds emitted by brown seaweed, *S. cinctum* that repel female corn borer moths.

Sargassum seaweed can be used together with other effective IPM strategies, especially in coastal areas, to reduce corn borer population. Brown seaweed can be an effective pest management strategy tool for low input and/or organic green corn production.

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