ARPN Journal of Science and Technology

Vol. 4, No. 3 March 2014

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Accepted Refereed Research Articles

ARPN Journal of Science and Technology >> Volume 4, Issue 3, March 2014

ARPN Journal of Science and Technology

Activity of Coal Fly Ash on Nilaparvata lugens:Disruption of Spiracle Function

Full Text	Pdf
Author	Hafiz Fauzana, F.X. Wagiman, Edhi Martono
ISSN	2225-7217
On Pages	209-216
Volume No.	4
Issue No.	3
Issue Date	April 01, 2014
Publishing Date	April 01, 2014
Keywords	coal fly ash, activity, brown planthopper, spiracles and respiratory function.

Abstract

Coal fly ash is potential as an insecticide to control brown planthopper (BPH), Nilaparvata lugens (Stal) through the disruption of spiracle function. This activity is related to the size of both coal fly ash particle and BPH spiracle. We conducted a laboratory study to determine the activity of the coal fly ash in disrupting BPH's spiracle function. The research was conducted to determine the activity of the coal fly ash in disrupting function of BPH spiracles. BPH body was treated with coal fly ash, and its spiracles were then photographed using Scanning Electron Microscope (SEM). We found that coal fly ash particle was resembling spheres of various sizes, ranging from 0.81 to 59.6µ. Most of these particles were <10µ (71.97%) and 10-20µ (20.68%) in diameter, while the remaining 7.35% were between with >20-60µ. The BPH spiracles were elliptical in shape and also vary in size, with the length and width ranging from 6.99 to 178.0µ and 5.72 to 63.76µ respectively. A significant amount of coal fly ash particles were able to BPH spiracles. Image analysis showed that all spiracles were covered by coal fly ash. Thoracic spiracles were sealed between 40-100%, while 95-100% of the abdominal spiracles were also closed. The covering of spiracles by coal fly ash interfered with the respiratory function of BPH and caused 71% mortality within 72 hours after treatment.

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Activity of Coal Fly Ash on Nilaparvata lugens:Disruption of Spiracle Function

Published in AJST, Vol. 4, No.3, March 2014

Saeed

Saeed Ullah
Managing Editor

ARPN Journal of Science and Technology
http://www.ejournalofscience.org

Vol. 4, No. 3 March 2014 ISSN 2225-7217
ARPN Journal of Science and Technology

http://www.ejournalofscience.org

Activity of Coal Fly Ash on *Nilaparvata lugens*:Disruption of Spiracle Function

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ABSTRACT

Coal fly ash is potential as an insecticide to control brown planthopper (BPH), *Nilaparvata lugens* (Stal) through the disruption of spiracle function. This activity is related to the size of both coal fly ash particle and BPH spiracle. We conducted a laboratory study to determine the activity of the coal fly ash in disrupting BPH's spiracle function. The research was conducted to determine the activity of the coal fly ash in disrupting function of BPH spiracles. BPH body was treated with coal fly ash, and its spiracles were then photographed using Scanning Electron Microscope (SEM). We found that coal fly ash particle was resembling **spheres of** various sizes, ranging from 0.81 to 59.6µ. Most of these particles were <10µ (71.97%) and 10-20µ (20.68%) in diameter, while the remaining 7.35% were between with >20-60µ. The BPH spiracles were elliptical in shape and also vary in size, with the length and width ranging from 6.99 to 178.0µ and 5.72 to 63.76µ respectively. A significant amount of coal fly ash particles were able to BPH spiracles. Image analysis showed that all spiracles were covered by coal fly ash. Thoracic spiracles were sealed between 40-100%, while 95-100% of the abdominal spiracles were also closed. The covering of spiracles by coal fly ash interfered with the respiratory function of BPH and caused 71% mortality within 72 hours after treatment.

Keywords: *coal fly ash, activity, brown planthopper, spiracles and respiratory function.*

1. INTRODUCTION

Brown planthopper (BPH), *Nilaparvata lugens* (Stal.) (Hemiptera; Delphacidae) is a major pest of rice in Indonesia that can cause harvest failure. The increase in BPH attack occurred in East, Central and West Java during the rainy season of 2009/2010 and in the beginning of the 2010 dry season [1]. A common way to control was the application of insecticides. However, this can lead to pest resistance and resurgence [2;3], therefore, it is necessary to search for an innovative method in controlling the BPH, such as the use of coal fly ash.

The activity of the coal fly ash has been studied in various insect pests species. More than 50 species of major crops insect pests were susceptible to various treatments of coal fly ash. The use of coal fly ash to control major pests of rice was effective. The coal fly ash caused damage to the mandible of chewing insects and interfered with the digestive tract. The damaged mandible caused insects to stop feeding and eventually died due to starvation [4;5].

On BPH, the particles of coal fly ash may interfere with spiracles function as these holes supported the exchange of O_2 and CO_2. BPH has a pair of spiracles on each thoracic and abdominal segment. Each of the holes was associated with tracheal spiracles [6]. The oxygen enters the spiracles and is transferred into the insect body through atracheal tube and tracheoles and reach dysfunction cells of the body. The CO_2 coming out of the cells follows the same path through the tracheoles into the trachea and through the holes to the spiracles and go out of the body [7]. The coal fly ash treated BPH died within a few hours after application [8], however the killing mechanism of these particles on this insects has no been studied. Therefore we conducted a study to determine the spiracles dysfunction due to treatment of the coal fly ash.

2. MATERIALS AND METHODS

The Preparation of the Test Insects

The test insects were brachypterous BPH obtained from rice fields in Yogyakarta and then reproduced at the Center for Biological Resource Management of Gadjah Mada University. One week old seedling of IR 64 rice variety were used as the host of BPH. The BPHs were cultured on the rice seedlings and then allowed to grow and multiply to sufficient number for study treatment. Regeneration was continuously conducted once a week to multiply the BPHs.

The Measurement of Coal Fly Ash

The coal fly ash was obtained from PT. PLN Pembangkitan Jepara of Central Java. The measurement of the coal fly ash particles was conducted using Scanning Electron Microscopy (SEM) JEOL JSM T300 brands at 2000 times magnification. The preparation of the coal fly ash samples was as follows: the samples were placed on the grid and thenglued together using carbon tape and placed on the holder. Samples were then coated with gold material using a fine coation sputter. Grid number three units. Fine coat ion sputter heated for 10 minutes, the grid holders put in fine coation sputter and appliances witched on for 15 minutes. The coated samples were poured into the column on the stage in the SEM tool and kept in vacuum until the indicator showed ready position. Coal fly ash particles were observed by adjusting them to the desired position in the control at 2000 times magnification. Image recording was conducted in five different views, they were; center position, the top left and right edges, and left bottom and right edges. The

pictures of the coal fly ash particles were subsequenttly measured using semaphore software. The recording was conducted on 2 other grids to obtain 15 images. Six best pictures were selected from 15 images. The particles were then classified to <10 μ, 10-20μ, >20-30μ, >30-40μ, >40-50μ and >50-60 μ.

The Measurement of BPH Spiracle

The measurement of BPH spiracle was conducted using Scanning Electron Microscopy (SEM) JEOL JSM T300 brands at 2000 times magnification. As many as 45 brachypterous BPH were killed with chloroform. There were totally 10 grids and on each of them laid three BPHs. Additionaly, there were 5 grids and on each of them laid 3 BPHs. Each of the specimens on the grids was bonded with carbon tape and placed in the holder. Furthermore, the BPH specimens coated with gold in a plating process as coal fly ash samples.

There were totally 45 brachypterous BPHs consisting of 30 individuals on left lateral position and 15 individuals on right lateral position and each of the segments of the spiracles were observed from the positions. The observation is focused on spiracle shape and size on each segment of both thorax and abdomen. The observable spiracles were then photographed and measured using semaphore software. Each of the spiracles on each of the segments either at right or left position in the thorax and abdomen images represented three of the most obvious ones.

The Observation of BPH Spiracle Treated with Coal Fly Ash

Thirty 30 brachypterous BPH were treated with coal fly ash in a plastic cup of 10 and 15 cm in base and top diameter respectively, and 25 cm in height. As much as 0.48 g/cup or 12 g/m^2 of coal fly ash was blown into the cup using aquarium water pump. After one hour, the test insects were killed with chloroform. Three BPH specimens were placed on the grid in the left lateral position, glued together with carbon tape, while there were totally 5 grids. Another 3 specimens of the BPH were placed on the grid in right lateral while there were totally 4 grids. The following grid was placed on the specimen holder and the BPH specimen was subsequently coated with gold in the gold plating process with the same process for coal fly ash samples. Subsequently, the SEM specimens with the BPH treated with the coal fly ash were observed at the magnification of 2000 times. Six BPH specimens were then observed under SEM 2000 magnification. The proportion of the coal fly ash covered spiracle was observed. The most obvious spiracles were recorded and measured using semaphore software.

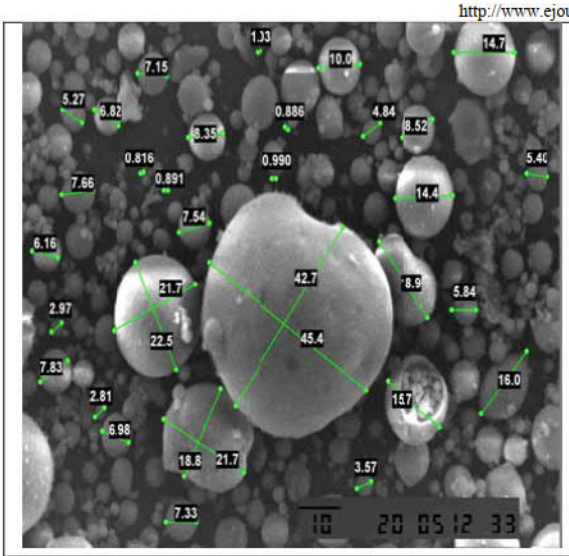
The Effect of Coal Fly Ash on BPH Mortality

The effect of coal fly ash on brachypterous BPH was measured by comparing the mortality of BPH treated coal fly ash and control. Five replications were employed. Twenty brachypterous BPH were maintained in 250 ml plastic cup with 2 weeks old rice seedlings. The cup was closed with gauze to prevent the BPH escape. An aquarium water pump was used to blow coal fly ash into the cups at the dose of 0.48g/cup or 12g/m^2 [8]. Number of dead BPH was recorded every 24 hours, until 72 hours after treatment.

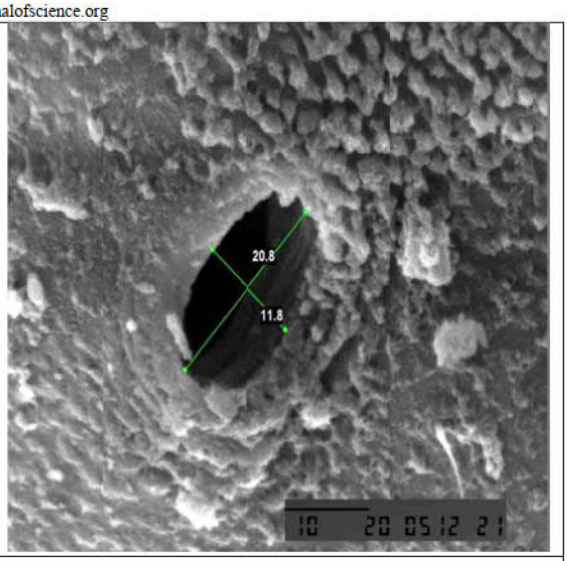
3. RESULTS AND DISCUSSIONS

The Comparison of the Spiracles and the Coal Fly Ash Particle Size

The coal fly ash particles were spherical like ball with diameter varied from 0.81μ to 59.6μ (variance coeff. 91.98). Most of the particles were <10μ (7.79%), 20.68% were 10-20μ, 4.52% were >20-30μ, 1.7% were >30-40μ, 0.27% were >40-50μ, and 0.27% were >50-60μ. The variation of coal fly ash particle diameter and shape was presented in Figure 1, and Table 1 and 2.



The coal fly ash particle mass



The spiracle of second abdominal segment

Figure-1: Coal fly ash particleand BPH abdominal spiracle size (SEM at 2000 X)

Table-1: The comparison of coal fly ash particle and BPH spiracle size

Statistics	Coal fly ash	BPH spiracle
Number of samples (n)	353	54
The smallest size (μ)	0.81	6.99
Largest size (μ)	59.60	178.00
Mean	8.32	34.69
S.D.	7.66	34.48
C.V.	91.98	99.38
T test α0,05	P = 0.00000075067 **)	

Table-2: The diameter of coal fly ash particles

Viewing Field	The Amount of Coal Fly Ash Particle of Size:						Total
	<10 μ	10 – 20 μ	>20 – 30 μ	>30 - 40 μ	>40 - 50 μ	>50 - 60 μ	
1	58	12	3	0	0	0	74
2	44	13	5	1	0	0	63
3	36	13	1	3	0	0	53
4	46	21	3	0	0	0	70
5	34	9	2	2	0	1	47
6	36	7	2	0	1	0	46
Mean	42.33	12.5	2.66	1.00	0.16	0.16	353
Mean Percentage (%)	71.97	21.25	4.52	1.7	0.27	0.27	100

Both BPH thoracic and abdominal spiracles were ellips-shaped with various sizes (Figure 1; Table 1, 3) ranging from 6.99 to 178.00μ, with the average size of 34.69x17.4μ (variance coefficient of 99.38).

The size of coal fly ash particles determine whether it could enter spiracle's hole and proceed to the tube tracheal. The average size of BPH spiracle were 4 times greater than the mean diameter of coal fly ash (Table 1).

Table-3: The size of thoracic and abdominal spiracle of brachypterous BPH

Body parts	Segments	Specimen	Spiracles Size (μ)			
			Length		Width	
			Right	Left	Right	Left
Thoracic	1	1	19.1	12.1	10.2	7.42
		2	15.6	54.0	6.3	32.2
		3	30.0	27.7	10.9	15.3
	2	1	48.9	25.6	30.9	16.5
		2	30.5	58.4	10.8	36.8
		3	33.2	37.4	8.7	29.4
	3	1	129.0	69.2	62.0	55.6
		2	178.0	61.1	72.0	34.8
		3	137.0	137.0	57.3	79.9
Abdominal	1	1	55.1	24.4	21.2	14.3
		2	21.3	23.1	12.7	12.7
		3	16.8	22.3	9.18	10.5
	2	1	25.0	20.8	12.3	11.8
		2	21.6	23.8	11.7	15.2
		3	16.7	19.9	10.7	10.8
	3	1	21.4	19.7	13.3	9.01
		2	20.4	17.5	8.07	11.0
		3	8.9	18.9	4.3	10.5
	4	1	33.7	17.3	11.5	5.48
		2	19.5	17.5	7.1	11.3
		3	23.0	20.1	10.8	8.7
	5	1	19.4	13.1	5.5	5.5
		2	18.5	22.6	7.9	13.9
		3	19.7	21.6	7.9	6.3
	6	1	6.9	23.0	4.56	10.8
		2	21.9	27.6	8.4	11.6
		3	22.3	24.1	4.21	9.07
Mean			37.5	31.8	16.3	18.4
S.D.			41.7	25.8	18.0	17.0
C.V.			111.1	81.0	110.5	92.2
tα0,05			P = 0.9364 NS		P = 0.0921 NS	

Note: Data were transformed using log (x), NS is not-significant

The spiracle size of left and right side of each thoracic and abdominal segment of BPH was also various (Table 2), eventhough they supposed to be similar for they were laterally symmetrical. This might be due to Technical error may cause mistake in sizing due to the specimens preparations and placing.

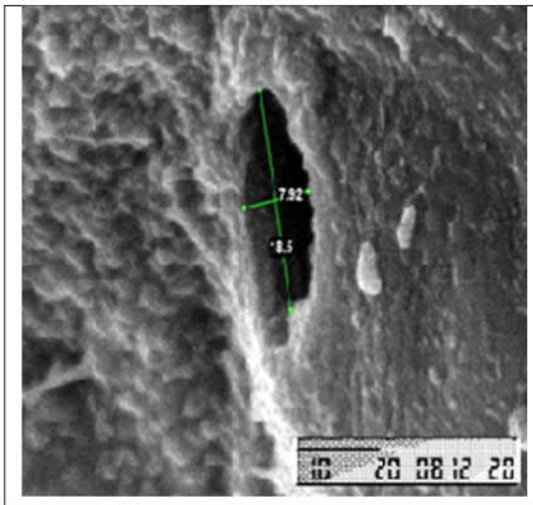
Generally, the size of thoracic spiracles was larger than that of the abdominal spiracles. The right and left spiracles of abdominal segments 1, 2, 3, 4, 5, and 6 were varying. The mean size of the right spiracles was 148.0 x 63.56 µ, and 89.1 x 56.76 µ for the left one, while mesothoracic and prothoracic segments where the smallest size among the spiracles was found on the thoracic segment (Table 3 and Figure 3).

The Covering of BPH Spiracles by Coal Fly Ash

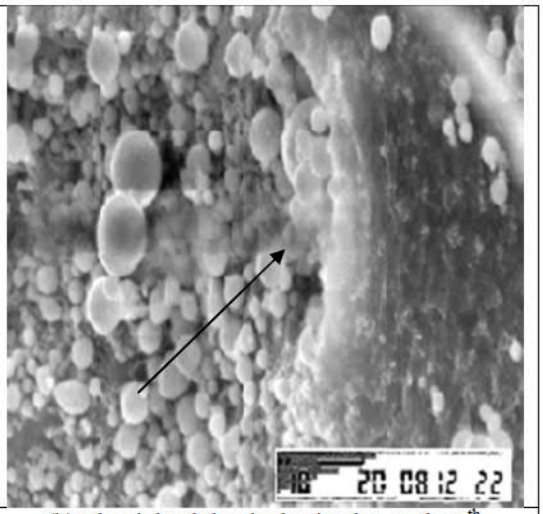
BPH spiracles were covered by coal fly ash after the dusting. This was obvious under 2000 X SEM magnification. The comparison and treated BPH spiracles was given in (Table 3).

Table-4: The percentage of BPH spiracles blocked by coal fly ash particles

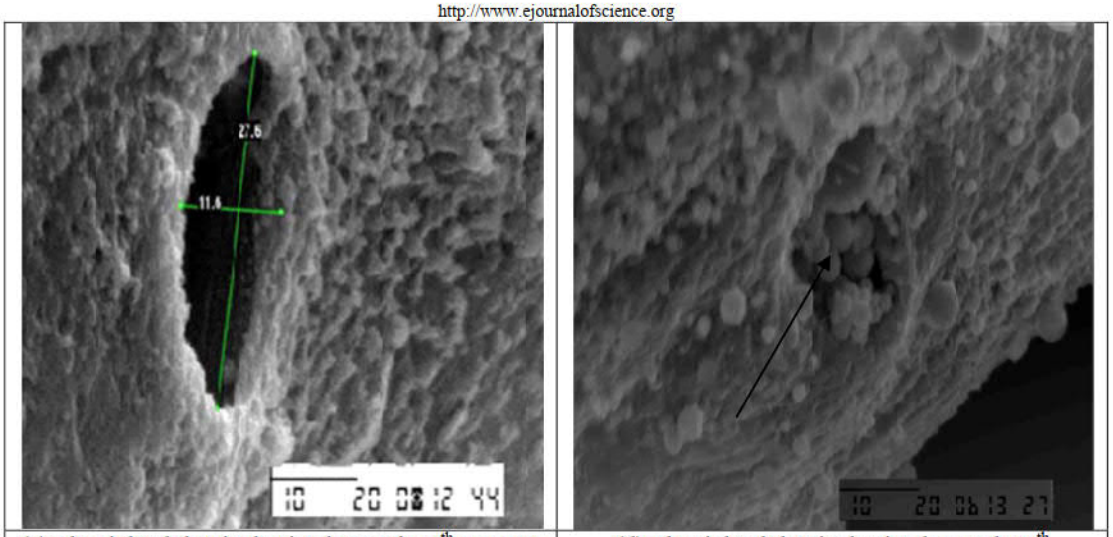
BPH Body Segments	The covering of BPH spiracle by coal fly ash (%)							
	Abdominal Segment				Thoracic Segment			
	N	Right	n	Left	n	Right	n	Left
Segment 1	3	100	3	100	3	100	3	100
Segment 2	3	100	3	100	33	40	3	70
Segment 3	3	100	3	100		50	3	50
Segment 4	3	100	3	100		-		-
Segment 5	3	100	3	95		-		-
Segment 6	3	100	3	95		-		-
Mean		100		98.33		63.33		73.33



(a) The 5th segment of right abdominal spiracle of control BPH



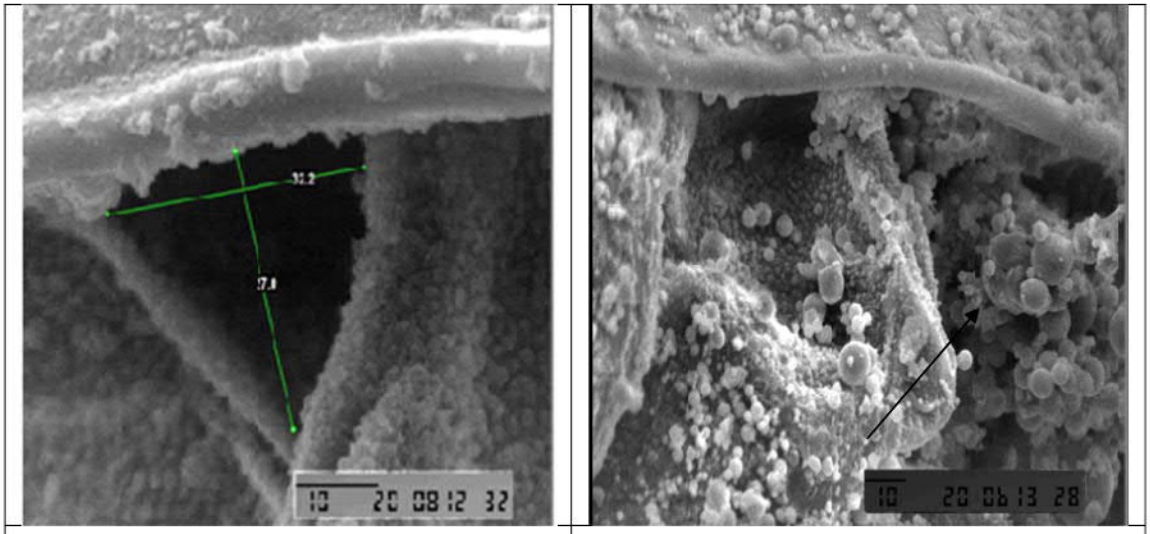
(b) The right abdominal spiracles on the 5th segments treated with coal fly ash



(c) The right abdominal spiracles on the 6th segment in untreated

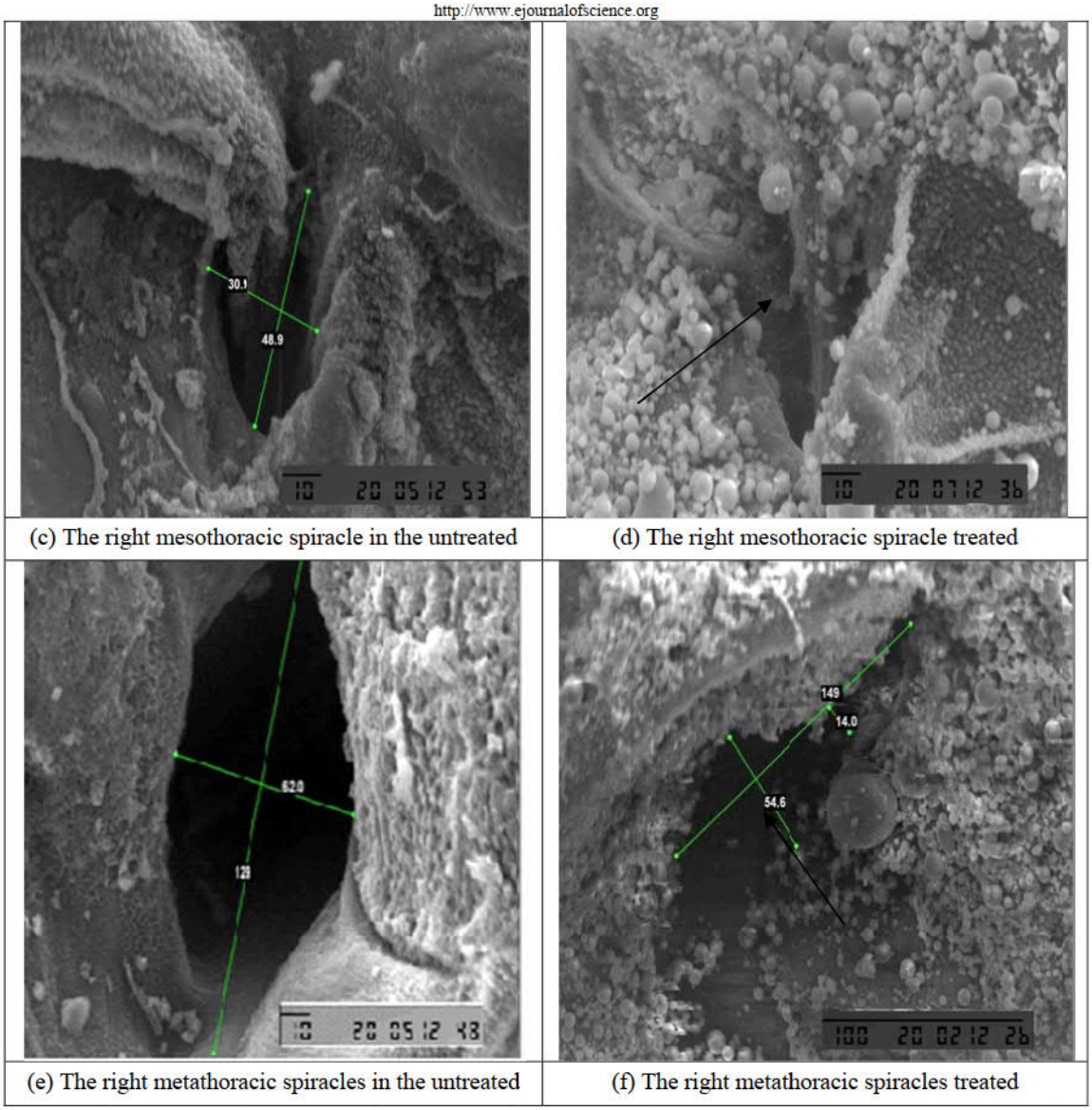
(d) The right abdominal spiracles on the 6th segments treated with coal fly ash

Figure-2: SEM of treated with coal fly ash and untreated BPH abdominal spiracles (2000X SEM magnification)



(a) The right prothoracic spiracle in the untreated

(b) The right prothoracic spiracle treated



(c) The right mesothoracic spiracle in the untreated

(d) The right mesothoracic spiracle treated

(e) The right metathoracic spiracles in the untreated

(f) The right metathoracic spiracles treated

Figure-3: SEM of treated with coal fly ash and untreated BPH thoracic spiracles (2000X SEM magnification)

All BPH spiracles of both thoracic and abdominal were covered by coal fly ash particles (Table 4). Spiracles holes on control and treated BPH were illustrated in Figure 2 and 3. Coal fly ash particle with the size of <30µ was succeeded to enter the BPH spiracles opening.

At normal conditions spiracles open and close the valve regulated by. In dry conditions where dust or other foreign material in, will be blocked by the hairs spiracles [3]. Therefore, coal fly ash particles were exposed to BPH in large quantities so that the hairs spiracles function was reduced to prevent then try of foreign objects and particles of coal fly ash into the hole spiracles. The proportion of spiracles were covered pit coal fly ash approximately 40-100% depending on the body segment BPH (Table 3). Although the hole closed spiracles not 100%, coal fly ash particles into the tracheal tube allegedly damaging a significant effect on the respiratory system.

The Effect of the Coal Fly Ash on BPH Mortality

Coal fly ash activity on BPH was insecticidal in nature. The mortality of brachypterous BPH treated with the coal fly ash reached 71% at 72 hours after exposure, while no deaths were found in control BPH (Table 4).

Table-5: The mortality of brachypterous BPH in control and treated cups

Replication	The mortality of brachypterous BPH (%)					
	24 hours		48 hours		72 hours	
	Treatment	Control	Treatment	Control	Treatment	Control
1	65	0	70	0	70	0
2	60	0	70	0	80	0
3	60	0	60	0	60	0
4	55	0	55	0	55	0
5	90	0	90	0	90	0
Mean	65.00	0	69.00	0	71.00	0
s.d.	10.48		12.00		12.80	
c.v.	16.13		17.39		18.03	

The spiracles were parts of the respiratory system of the insects (i.e., the ingress of oxygen and release of carbon dioxide) and if the covering occurred, it caused the reduction of the oxygen intake so that the insects would experience respiratory problems and it could lead to death [9]. The covering of the spiracles of the BPH pest in the abdominal and thoracic parts with the coal fly ash particles reduced the supply of O_2 and inhibited the release of CO_2. The BPH deaths due to the coal fly ash treatment allegedly took place because of respiratory disorders.

4. CONCLUSION

Coal fly ash activity on BPH was showing the insecticidal nature. At the dose of 0.48 g/cup, BPH mortality reached 71% at 72 hours after application. There were 40-100% of the BPH deaths due to the coal fly ash treatment related to respiratory dysfunction, due to the covering of the spiracles.

ACKNOWLEDGEMENTS

The author would like to express sincere gratitude to the Directorate General of Higher Education that has provided the grant through the Graduate Research Program of Riau University for doctoral students.

Author's special thanks to the Borobudur Heritage Laboratories Magelang for the SEM facility especially the technical assistance of Mr. Puji Santosa.

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