

The Combination of Piper *Caninum Blume* Leaf Extract and Compost Fertilizer for Pressing Blast Disease and Improving Growth of Bali Red Rice (*Oryza Sativa Linn*)

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Abstract— Blast disease is a spotting disease on the leaves that attacks rice, rice grains become empty and finally causing a significant loss in rice production. Many factors that cause blast disease include the continuous use of synthetic fertilizers and pesticides which could decrease soil fertility resulting in a decrease in rice yield. One way to restore soil fertility and increase rice production is to use organic methods such as using biopesticides and compost. The purpose of this study is to obtain the most appropriate formulation between biopesticides, *P. caninum* BL. leaf extract and compost to suppress blast disease and improve results in red Bali rice cultivation. This research was conducted in the field in Senganan Village, Penebel, Tabanan Bali, a flat area of about 300m above sea level. The experiment was carried out using a randomized block design (RBD) with 4 set of different treatments. The mixture of 40% compost and 2% piper caninum Bl. leaf extract was found to be the most effective formulation to suppress blast disease by 57.17% when tested in a 0.2-hectare paddy field. The significant reduction of blast disease and the use of organic fertilizer have improved the growth of red rice as shown by the average number of tillers (12.9) and leaves (60.5 strands) after 12 weeks of transplant. The yield of the rice after 3 months of cultivation was 5.51 ton / ha, an increase by 2.40%. The morphology of the ripen grain of F4 treated clumps were studied and EDX analysis showed only carbon and oxygen are the major elements and trace amount of potassium and magnesium were detected. The crystalline nature of the rice is shown by the presence of polyhedral shape of starch with the size between 3 and 6 μm in the endosperm area. The silicon was only observed in the hull layer. At an optimal extract concentration, the yield was increased significantly by 77%. However, above the optimal concentration of the extract toxic behaviour was observed in both the growth and reduction of yield. This may be due to the toxicity nature of the many active components that were present in the extract. Therefore, further study is necessary to understand the mode of action and side effects that enable to assist in the modelling work for cultivation management and practices.

Keywords— Bali red rice; organic; compost; extract; *P.caninum*; morphology; blast.

I. INTRODUCTION

Blast disease caused by *P. oryzae* fungus [1] on rice cultivation is not new. However, the fact that it can infect rice plants in various stages of growth and could cause between 30 and 50% loss of production in endemic areas is a serious matter [2]. Although proper NPK fertilization can reduce the attack of blight-causing fungi the control of the disease is not always easy. High concentration of nitrogen would also increase the chance for the attack. Therefore an effort to overcome the disease on rice has been the subject of research for quite some times. Rice blast forecasting model

has also been studied for application by the farmers in their planning stage [3], [4]. The use of synthetic fungicides although effective [5], [6], for longer period of time and inappropriate way of application can cause a negative impact on the rice crop ecosystem. Planting resistant rice varieties in alternating manner to confuse the fungi has been fairly successful [7]–[10]. The use of antagonistic microbes such as *Enterobacter agglomerans*, *Serratia liquefaciens* and *Xanthomonas lumenescens* has shown an improvement between 22 and 52 % [11]. On the other hand *Trichoderma harzianum* was found to show higher inhibition of 71-88% growth of mycelia and spores from *P. oryzae* fungi [12], [13]. In another study at Banyuasin district, a compost

extract was found to increase rice yields and suppressed 60% blast disease [14]. *In vivo* study in our laboratory using leaf extract of *P. Cinanum* also showed high inhibitory activity of 83.3% against blast disease at 2.5% concentration [15]. *P. Cinanum* is known to contain biologically active compounds against microbes including bacteria and fungi [16]. Based on this study we decided to test the effectiveness of the extract in combination with organic compost fertilizer in the cultivation field with an idea of dual functions to control the blast disease and supporting the growth of the plant as well. The optimal formulation will be useful for wider applications as bio-pesticide.

II. MATERIALS AND METHODS

A. Experimental cultivation design

Experimental cultivation of rice was carried out in the field at Tabanan West Bali area (longitude 115.0; latitude - 8.45 and elevation 249m above sea level) based on Group Random Design with 2 factors, 5 treatments and 4 replications. Altogether there are 20 experimental units and each unit consists of 5 clumps with the size of 30x30 cm. Each clump will take only 2 seeds. The size of each unit is 150x150 cm. The average annual temperature is 25.9°C and annual rainfall of 2178mm. The cultivation experiment was conducted in March till July 2018. Each clump in the unit is labelled as F0 for control; F1 = treatment with (10% compost + extract 0.5% mixture); F2 = treatment with (20% compost + 1% extract mixture); F3 = treatment (30% compost + extract 1.5% mixture) and F4 = treatment with (40% compost + 2% extract mixture). The nature of the soil was sandy loam. Standard cultivation steps involving seed seeding, planting media preparation, seed planting, plant maintenance and inoculation of pathogenic fungi (*P. orate*).

B. Scanning Electron Microscopy

The grain rice was cut longitudinally or transversally and mounted on a circular aluminium stub and platinum-coated in a vacuum using a sputter coater. The rice morphology was examined by using scanning electron microscope energy-dispersive X-ray spectrometry *FE-SEM, ZEISS Merlin operating* at 0.2-30kV beam current up to 400nA and the lowest vacuum of few pA-300nA. In the present study 3kV accelerating electron was applied for the imaging and 15kV for EDX experiment except at the husk where 10keV was sufficient.

C. Extraction of *P.caninum* Blume

Forest chili leaves (*P. caninum* Blume) were collected from plants grown in the Village of Senganan, Penebel District, Tabanan Regency, Bali. The first three matured leaves from branch end were collected because the 4th leaf and beyond contained less active components and therefore become less active. The collected leaves were washed with clean water to remove contaminants, then cut to small pieces and wind dried for 3 days in the shade. The materials (1kg) were then macerated in methanol at a ratio of 1:10 (weight / volume) for 48 hours in the dark, at room temperature. The filtrate was obtained by filtering, using 4 layers of gauze followed by filtration using *Whatman* filter paper No. 1. The maceration process was repeated 3 times with methanol. The

filtrate obtained were combined and then evaporated using a rotary evaporator at 40° C to remove the solvent (methanol). 10g crude gel-like product was obtained.

D. Organic compost Fertilizer

The fertilizer was prepared by mixing appropriate amount of cow dung, manure chicken, rice straw and *trichoderma* bio-starter either isolated from local soil or as supplied by government agency and *aspergillus niger* in a container. Then small amount of water was added to make the total weight about 200kg. The container was closed for 20 days. The container was opened to release the gas and the whole mixture was thoroughly stirred. The container was closed again for 40 days after which it is ready for use [17].

E. Preparation of organic pesticide-fertilizer mixtures

Four different ratios of fertilizer-extract mixtures were prepared in 100mL each. The first mixture for the first clamps is labelled as F1 has 10% compost and 0.5% extract. 10g compost and 0.5g extract were weighed and mixed in 200mL container. 100 mL of water was added to the mixture and the mixture was stirred thoroughly. Similar procedure was repeated for the 2nd clamp F2 (20g compost, 1g extract), 3rd clamp F3 (30g compost, 1.5 extract) and 4th clamp F4 (40g compost, 2g extract).

F. Application of Pesticide-Fertilizer Mixtures

The 20 days old germinated seed was transplanted into the clamps or unit plots. The water level was kept about 2cm above soil level. After transplanting of seeds into the clamps of the unit plots the young germinated seed (about 15cm height) were allowed to grow by maintaining the water flow through the well-known world heritage Subak system. The blast microbe *pyricularia oryzae* solution (20mL) was sprayed over the growing rice after 29 days of transplant. One day after, 20 mL mixed fertilizer-*P. cinanum* extract was sprayed on each clamp according to their respective treatment formulation (F1-F4). The same fertilizer-extract solution treatments were given to the growing paddy every 30 days until the fourth month. In total 4 treatments of fertilizer-extract solution were applied to the plants until harvesting stage.

G. Parameters observed

Observed and measured parameters include blast disease intensity and growth parameters (plant height, number of leaves and number of tillers). The intensity of blast disease was calculated by the following formula.

$$IP = \frac{\sum_{i=0}^i (nivi)}{NV} \times 100 \quad (1)$$

Where, IP=blast intensity, ni= number of leaves with a score to i, vi = Value of each category of disease scores, N = number of leaves observed, V = highest score.

Score for each category:

- 0 = no attack at all
- 1 = very mild attack (0-10% broken leaf surface)
- 2 = mild attack (10-30% broken leaf surface)
- 3 = moderate attack (30-50% of the leaf surface is damaged)

4 = severe attack (50 - 75% of the leaf surface is damaged)
 5 = very heavy attack (75 -100% of the leaf surface is damaged)

The inhibitory activity was obtained by dividing the difference between the number of blast of treated clamp and the control F0 by the number of blast of F0 treated clamp and multiply by 100.

H. Data Analysis

The data obtained were analyzed quantitatively using analysis of variance one way ANOVA ($p < 0.05$) and expressed as mean values. Statistical package SSPS Version H.O was used. If the treatment causes a difference to the observed variables, then Duncan's Multiple Range Test (DMRT) at the 5% level was used.

III. RESULTS AND DISCUSSION

A. Intensity of Blast disease

The field observation clearly showed that the control clumps were very much affected by the blast diseases (Fig.1).



Fig. 1. The effect of blast on the leaves in the control clumps (Fo)

However, significant improvement or reduction of blast intensity was displayed by the fourth clumps F4 (Fig. 2). Table 1 shows the intensity of blast disease and inhibiting activity after 9 weeks of transplanting. At this time the green paddy started to appear. The highest intensity was displayed by control unit (F0). The decrease of intensity as the percentage of fertilizer-extract increases indicates the positive effect of the dual fertilizer-pesticide. The lowest intensity (9%) with the highest inhibition activity (57.2%) was displayed by formulation F4. It is a very significant cure if not all.



Fig. 2. Less number of blast in the F4 treated clumps

TABLE I
 INTENSITY OF BLAST DISEASE AND INHIBITING ACTIVITY AFTER 9 WEEKS OF TRANSPLANT

No	Treatment	Intensity of blast disease (%)	Inhibiton activity (%)
1	Fo	66.18±0.01	-
2	F1	44.20±0.00	33.2
3	F2	24.20±0.05	63.6
4	F3	10.30±0.03	84.4
5	F4	9.01±0.01	86.4

The trend of change of both blast intensity and inhibition activity against the concentration of the extract is apparently linear at the beginning looked closer to exponential within the standard error towards the end as shown in Fig. 3.

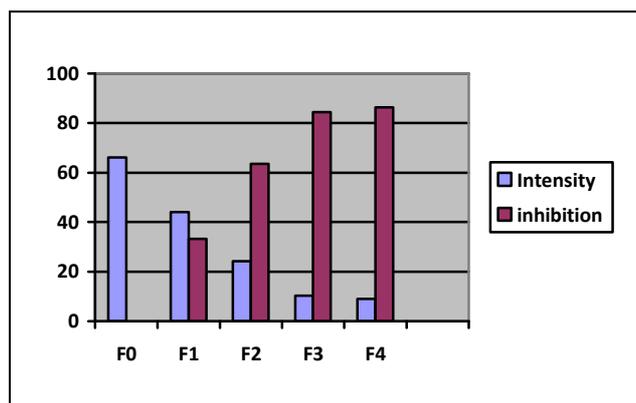


Fig.3 The relationship of intensity of blast disease and inhibitory activity with the increasing fertilizer-extract concentration

The effectiveness of the treatment is displayed by further decrease of intensity of blast disease after 15 weeks, close to the harvesting stage (Table 2).

TABLE II
 INTENSITY OF BLAST DISEASE AND INHIBITION ACTIVITY AFTER 15 WEEK

No	Treatment	Intensity of blast disease (%)	Inhibition activity (%)
1	Fo	79.11±0.12	-
2	F1	30.17±0.48	60.1
3	F2	20.15±0.01	74.5
4	F3	8.39±0.01	89.4
5	F4	7.11±0.05	91.0

Limiting the dosage of *P. caninum* extract up to 2% in the F4 formulation was based on our previous *in vitro* antifungal activity of *P. caninum* Blume extract against *pyricularia oryzae* and later study on its efficacy when applied to red rice plot in the laboratory glasshouse environment [18]. The *caninum* extract showed a strong inhibitory activity with inhibition zone of 44mm and MIC value of 0.5 % (w/v). In the efficacy study, 90.5% inhibition and 7.43 blast intensity were obtained at 2.5% *P.cinanium* extract and gave the highest grain yield of 11.43t/hactare but at 3.5% extract the intensity of blast disease increased to 12.93 and the yield decreased to 7.84 ton/hectare. The soil and urea fertilizer are not likely the main reasons for it.

Therefore, the plant extract at higher concentrations has become toxic to the plant. Chemical constituents in *P.caninum* plant has been studied quite extensively. Some

the number of leaves after the 12th week. This is due to aging after certain time.

TABLE IV
THE EFFECT OF FERTILIZER-EXTRACT MIXTURE FORMULATION ON THE AVERAGE NUMBER OF LEAF OF THE RICE PLANT

Fertilizer-extract mixture formulation	Leaf		
	Week (4,8,12)		
F0	28.8±0.17	45.5±0.40	35.1±0.58
F1	28±0.12	45.7±0.06	37.1±0.17
F2	30±0.12	47.5±0.23	40.8±3.1
F3	42±0.00	70.4±0.12	60.5±3.3
F4	43±0.29	65.4±0.03	60.5±1.0

The first four weeks the untreated control clamps showed a slow growth relative to the treated clamps (Table 5). However, they all reached about the same height in the 8th week. After 12 weeks surprisingly the control clamps showed slightly taller than the rest. In fact the height of F4 treated clamps is slightly shorter than the others. The final height between 167 and 180 cm seem acceptable under the current environment although height up to 200cm has been observed and at this height the tree might have bend and fell down to the ground.

TABLE V
THE EFFECT OF FERTILIZER-EXTRACT MIXTURE FORMULATION ON THE AVERAGE NUMBER OF HEIGHT OF RICE PLANT

Fertilizer-extract mixture formulation	Height (cm)		
	Week (4,8,12)		
F0	28.3±0.46	119.8±0.46	179.7±0.38
F1	39±0.58	122.1±0.067	176.6±0.08
F2	39.2±0.12	120.5±0.13	175.1±0.40
F3	40.7±0.12	116.4±0.49	172.4±0.72
F4	40.5±0.97	116.3±0.17	167.7±1.1

C. Crop and Yields

Despite the disease the plants still grow and reach a reasonable height and gave grains indicating the resistance capability of the brown rice plant against the infection. The untreated clamps F0 have the longest stem (179cm) but many of them have bent and felt to the ground (Fig. 5) compared to F4 treated (Fig.6) and the rest during the latter part of the ripening phase or harvesting stage. It is known that under proper cultivation condition, brown rice could reach 220cm but will certainly bend and fell to the ground during the later stage of the ripening phase due to overweight of the grain.

Although F0 treated clamps had the lowest tillers, the number of grain/panicle is also the lowest but not very much different than the treated clamps (Table 6). However, the percentage of empty grain in the control clamps is the highest (11.74%) while the F3 and F4 treated had only about 2.89%. F4 treated clamps again showed the highest full grain weight/clump. So, the infected clumps without treatment certainly have low yield.



Fig. 5. Control rice plant (F0) after ripening stage 14 week after transplant



Fig. 6 F4 treated clamps during harvesting time 14 week after transplant

TABLE VI
EFFECT OF LEAF EXTRACT TREATMENT ON YIELD COMPONENTS OF RICE PLANTS

Treatment	Number of productive tillers	Number of grain/panicle	Full grain weight/clump (gram)	Percentage of empty grain/clump (%)
Fo	8.5±0.5	218.81±0.81	41.60±0.23	11.74±0.3
F1	10.5±0.12	234.24±0.62	52.80±0.01	9.59±0.27
F2	12.5±0.29	237.50±0.23	60.91±0.40	7.62±0.31
F3	12.7±0.06	240.76±0.19	79.98±0.61	2.89±0.18
F4	12.9±0.94	240.78±0.21	80.23±0.07	2.75±0.14

It is clear that F4 treatment showed positive result with the highest increase of potential yield of 77.2% (Table 7). The size distribution of the grain was quite consistent (Table 8) with an average length-width ratio of 2.38 indicating that it could be classified as medium grade rice.

TABLE VII
POTENTIAL YIELD AND YIELD INCREASE

Treatment	Potential yield (ton/ha)	Yield increase compared with control (%)
F0	3.11±0.24	-
F1	3.78±0.36	21.5
F2	4.22±0.06	35.7
F3	5.44±0.09	74.9
F4	5.51±0.05	77.2

TABLE VIII
AVERAGE SIZE OF RED BALI RICE FOR EACH CLAMPS

Treatment	Long (mm)	Wide (mm)	Diameter (mm)
F0	0.51±0.06	0.20±0.01	0.3±0.02
F1	0.53±0.06	0.23±0.06	0.35±0.01
F2	0.60±0.17	0.25±0.06	0.35±0.05
F3	0.65±0.06	0.28±0.17	0.37±0.01
F4	0.70±0.8	0.31±0.06	0.42±0.01

D. Morphology of ripen rice grain by Scanning electron microscopy

It is not easy to see all sections or parts in a ripen rice grain clearly. Special technique of sampling preparation minimizing the structural damage has been developed by Kawamoto [26] called cryomicrotome enable to see all the parts including the soft and sensitive of the area such as embryo intact but in the process several slides and pictures are needed to look for the best one [27,28]. The best F4 treated clamp has the lowest empty grain per clamp (Table 6). Some filled grains without removing the hull were chosen and cut carefully, and the less damaged cross sections were selected for the morphology observation by using electron scanning microscopy. Fig.7 (a) shows the longitudinal cross-section of the rice grain with the upper ventral (Awn) on the left side and the dorsal part with the embryo on the right-hand side at 23x magnification. The outer most layer is the hull, and the upper part of the inner rice is almost separated from it with big empty-space in between. At 73X magnification at the upper part of the grain, we can see several layers after the hull toward inside the rice (Fig. 7b).

However, it is not easy to identify the pericarp, seed-coat, *nucellus* and *aleurone* layers. Unlike much-colored rice, the pericarp layer is easily distinguishable due to their anthocyanin color and can be removed, the color of the Bali red rice is homogenous in the whole body of the rice as shown in Figure 8. The *subaleurone* and inner endosperm are observed. The thickness of the hull is about 72 micron.

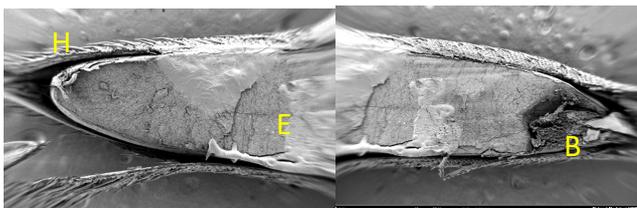


Fig. 7. (a) Longitudinal cross-section of red Bali rice at a 23X magnification at 3keV. E=endosperm; H= hull;B= Embryo

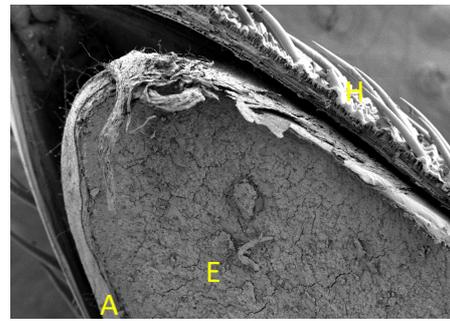


Fig. 7(b). Morphology of the upper part of the grain at 73-times magnification. H=hull; E= endosperm; A=Aleurone

At 500 time magnification, there are two layers probably Aleurone and *subaleurone* layers with a thickness of 20 and 7 microns respectively (Figure 7c).

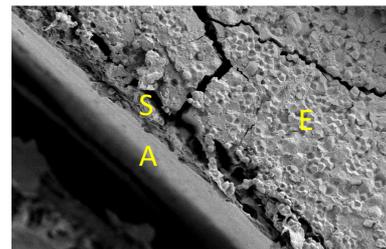


Fig. 7c. Micrograph showing the Aleurone and subaleurone layers at 500X magnification. A= Aleurone; SA= subaleurone; E=endosperm

In the endosperm area, the polyhedral starch crystals of size between 3and six μm were observed (Fig. 7d). There are also few crystals with spherical shape.

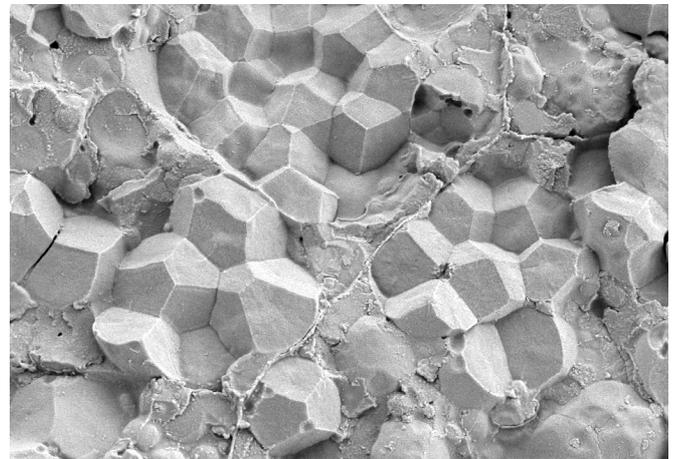


Fig. 7d. The polyhedral crystals of starch in the endosperm area at 3000X magnification



Fig. 8. Photograph of Padang (left) and Bali (right) red rice

E. Transversal cross section

In the transversal cross-section (Fig. 9a), we could see the individual aggregated polyhedral crystals as shown in the longitudinal cross-section is packed in blocks or cells of

different sizes and linked to one another and formed like a radial chain towards the center of endosperm.

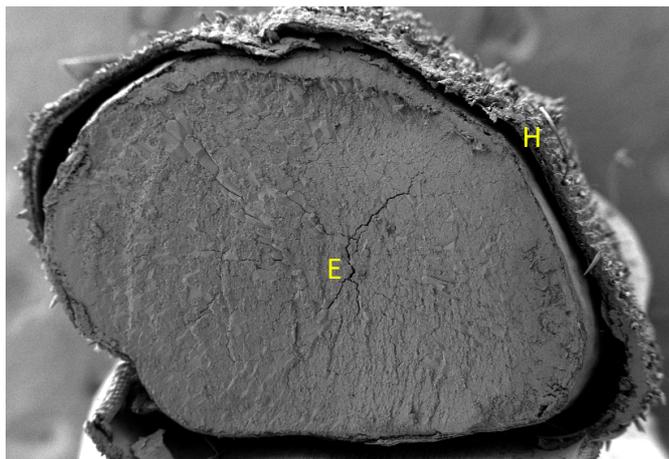


Fig. 9a. The transversal section of the Bali red rice at 39-time magnification. The outer most layer is the hull. H= hull; E=endosperm

There also individual crystals that are closely agglomerated located either between the blocks or sporadically distributed at the center of the endosperm (Fig. 9b). It is likely that the packing of crystallites occurred from the outer surface toward the center of the endosperm. The length of the block or cell is between 20 and 226 microns.

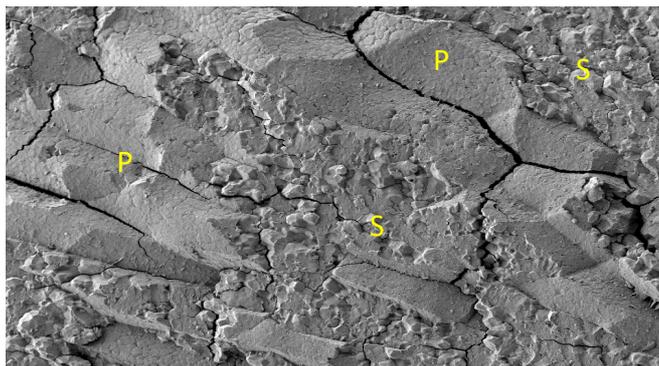


Fig. 9b. The micrograph near the central part of endosperm at 300-time magnification. P=block or cell; S=agglomerated crystals

F. Elemental Determination by Energy-Dispersive X-ray (EDX) Analysis

EDX is the easiest and quickest technique to detect elements and do semi-quantitative analysis. It may not be accurate if the presence of the elements is low below than 3% by weight. Carbon, oxygen, Na, Mg, Si, P, K, Ca and Al have been detected in the hull section of the rice grain [29]. However, at 15keV the Bali red rice hull showed only the presence of C (60.3%), O (26.5%) and Si (2.6%). K and Mg were detected in the area closed to the seed coat part of the grain as trace elements with a weight percentage of 1.6 and 0.5 respectively (Figure 10). Much higher content of Si and other elements were detected in conventional rice by XRF and ICP, neutron activation analysis (INAA), EDXRF methods [30], [31]. No silicon was detected in the internal part of the grain. The endosperm and bran area is carbon and oxygen only.

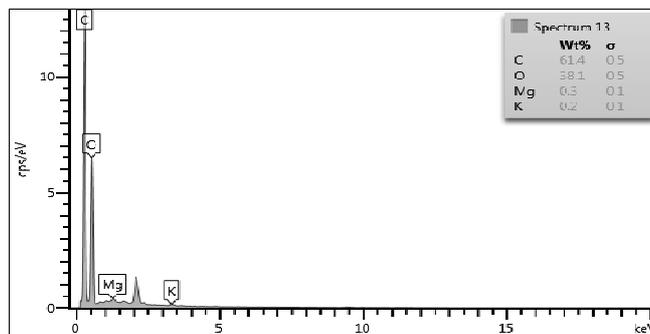


Fig. 10. EDX spectrum at the Aleuron area

The elemental mapping of the postural ventral part of rice grain (Fig. 11) shows the distribution of silicon (blue dots) only in the rice hull or husk.

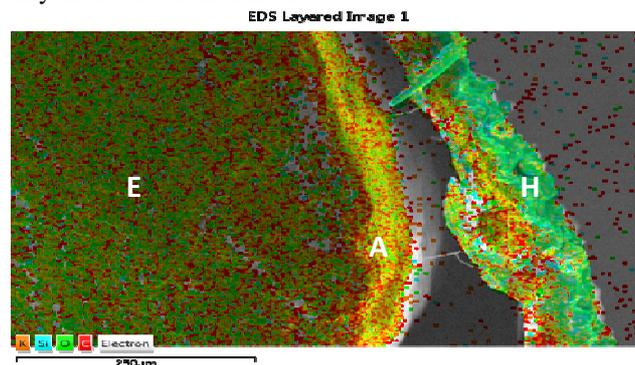


Fig. 11. Elemental mapping of the postural ventral part of the longitudinal cross section

IV. CONCLUSION

The studies demonstrated the application of organic compost-plant extract, in particular, *P.caninum* to combat blast disease and to promote the growth as well in rice cultivation in the field. At optimal extract concentration, the yield was increased significantly by 77%. However, above the optimal concentration toxicity behavior was observed in both the growth and yield. This may be due to the toxicity nature of the many components that were present in the extract. Therefore further study is necessary to understand the mode of action and side effects that enable to assist in the modeling work for cultivation management and practices.

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