BIOGENIC SILICA NANOPARTICLES, SYNTHESIS, CHARACTERIZATION AND ANTIFUNGAL ACTIVITY AGAINST TWO RICE PATHOGENIC FUNGI

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Abstract

Silica nanoparticles (Si NPs) were extracted by different methods from mrice husk (RH)and ric estraw (RS), and characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). For the XRD results, the crystalline size was calculated using Scherer equation. The particle size of rice husk nanoparticles (RHNPs) and rice straw nanoparticles (RSNPs) were 73.6 nm and 133.7 nm, respectively. Silica present in RHNPs and RSNPs was about 22.78 and 9.56%, respectively**.** Si NPs were effective in controlling rice blast (*Pyricularia grisea*) and brown spot (*Bipolaris oryzae*) fungal diseases under greenhouse conditions during 2015 season. Efficiency of soil application with RHNPs and white rice husk ash WRHA 92.56 & 90.90% at the rate of 1.5 g/1kg, were the most effective treatments to reduce blast disease severity compared to other treatments as well as control. On the other hand, rice plantswere treated with liquid potassium silicate (K_2SiO_2), WRHA and RHNPs gave 96.92, 93.07 and 91.53 % efficiency, respectively as foliar application for the control of brown spot disease compared with other treatments. SEM/energy-dispersive spectrometer (EDX) observations and X-ray spectra of adaxial surfaces of the fourth rice leaves Sakha 101 rice cultivar) in soil applied with 1.5g/1kg gave different types of silicified cells. The corresponding EDX spectra compared with the SEM images demonstrated differences in silicon content between soil treated by RHNPs 13.75% and non-treated plants 10.6%. Silicon accumulation in Sakha 101 rice leaves treated with RHNPs as soil application at 1.5g/1kg was increasing Si layers in epidermal cell walls, cuticle and the thickness of the silicon layer. Also, outer regions of epidermal cell walls and intercellular spaces within sub-epidermal tissues. All the silicon layers may be playinga role in increasing the resistanceof rice plants and controlling the rice diseases.

Keywords: Rice husk - straw- Silica– NPs- *Pyricularia grisea*-*Bipolaris oryzae*

INTRODUCTION

Rice diseases are one of the most limiting factors of rice production.In Egypt, blast disease caused by *P. grisea* Sace. is considered the most important disease affecting rice crop (Osman *et al.*, (2002), the second is brown spot caused by *B.oryzae* (Breada de Hann). The losses due to both diseases in developing countries

are estimated to be 60-80% higher than in industrialized countries (Vishunavat, 2012).Rice husks are the hard protecting coverings of grains of rice (Dominic *et al*., 2013). The main component of rice husk is silica (15 to 17 %) (Thudaji and Nuntiya, 2008). Simple sol get method was used to obtained silica NPs from rice husk. The synthesized RHNS was characterized by Fourier transform infrared (FTIR), XRD, SEM, TEM etc. The particle size of RHNPs was found to be 10-15nm (Dominic *et al*., 2013). Nano silica was prepared from rice husk with high surface area, and characterized by different method such as TEM images proved average size of 6 and 7 nm, respectively. X-ray diffraction pattern showed the amorphous form of produced silica (Ezzat and Shahebrahimi, 2012). Applications of silicon-containing fertilizers to paddy fields resulted in increased control of several economically important diseases such as blast (Seebold *et al.,* 2000), brown spot (Datnoff *et al*., 2007) with improved yield and quality. Adding silicon to plants as a fertilizer makes them more resistant to various pathogenic fungi (Datnoff *et al*., 2007). A 40% reduction in the incidence of neck blast on rice plants supplied with silicon was reported by Seebold *et al.* (2004). Application of 2mM silicon solution decreased the area under brown spot progress curve and the number of brown epidermal cells caused by *B. oryzae* on rice plants (Dallagnol *et al*., 2011). Osman *et al*. (2002) found that different sources and methods of silica application significantly decreased blast, brown spot and false smut severity.Silicon accumulation and locations played a role in resistance to rice blast disease as well as Electron-dense silicon layerswere frequently found beneath the cuticle in epidermal cell walls ofsilicon-treated plants,increasing levels of silicon were detected in theouter regions of epidermal cell walls, middle lamellaeand intercellular spaces within subepidermaltissues Kim *et al*. (2002).Rice diseases such as blast, brown spot and sheath blight becoming more severe on rice plants grown in Si-depleted soils. Si plays an active role in resistance of some plants to diseases and associated with the accumulation of phenolics and phytoalexins as well as with the activation of some PR-genes (Rodrigues and Datnoff, 2005). Silicaamount in the youngest rice leaves confers physiological resistance against blast infection after the penetration (Hayasaka *et al*., 2008).

The objectives of the current research were to synthesize and characterize Si NPs from RS and RH bio-waste using thermo-chemical methods. The antifungal activity of Si NPs with different concentrations was tested by using different application methods against rice blast and brown spot diseases in greenhouse assays. Another part of work was devoted to evaluate the mechanism of silicon induced resistance using scanning electron microscope and transmission electron microscope.

MATERIALS AND METHODS

1.Preparation and synthesis of Si NPs**:**

1.1. Samples of rice straw (Giza 178 cv)was collected and ground at Rice Pathology Lab., Rice Research & Training Center (RRTC), Sakha,Egypt. Theground rice straw powders were thoroughly washed in water to removedirt and aqueoussoluble substances by repeated vigorous stirringand decanting until the aqueous wash turned clear. The cleanedpowders were filtered and oven-dried at 70 °C for 72 h. The dry cleanrice straw powders wereheated in a muffle furnaceat three-stages, first to 250°C and held for 1 h,then to 325 °C and stayed for another 1 h, finally to 575°C and heldfor 3 h to remove the organics and resultant carbon. Extraction of silica from white rice straw ash5 g was dispersed in 500ml 0.5M NaOH aqueous solution and heated at 100 °C for 4 h under vigorous stirringto dissolve silica and produce sodium silicate. The resultant slurrywas filtered and washed with double distilled water (d.d.water) to remove nonreactive impurities.The transparent filtrate sodium silicate solution was allowed tocool to room temperature and was titrated with 10% H_2SO_4 to pH 7under vigorous stirring. Sodium silicate has shown to be neutralizedwith diluted sulfuric acid to precipitate silica. After neutralization,the solution was stirred for 24 h, and then set aside for another48 h to allow the silica gel to slowly precipitate. The formed gel was broken, filtered, and washed with d.d. water to remove sulfate salt untilthe conductivity of wash solution was below 200 S/m. The clean silica (RSNPs)gel was quickly frozen by liquid nitrogen and freeze-dried overnight toremove water. The freeze-dried product was stored in vacuum desiccatorsfor further characterizations (Ping and Hsieh, 2012).

1.2. Rice husks of (Giza 178 cv) were collected from a grain quality lab. At RRTC. It was thoroughly cleaned with tap water to remove adhering soil and dirt. Cleaned RH (100g) was refluxed with 1L, (1N) HCl at 90^0C for 1h to remove metallic impurities. After the reaction the acid was completely removed from the RH by washing with d.d.water. It was then dried overnight in an oven at 110°C.The treated RH was calcined in a muffle furnace at (700°C) for 4h. Silica with high degree of purity was obtained in the form of white ash (WRHA). WRHA (10g) is refluxed with 80ml of 2.5N NaOH solution for 4h. The resulting solution was continuously stirred under 70° C and filtered to obtain clear sodium silicate solution. Silica gel was produced by adding concentrated HCl drop wise to sodium silicate solution with constant stirring until pH equal to 9. The gel obtained was collected for 24h and washed with d.d.water, dried

in an oven at 70^0C for 24 h. The obtained Si NPs were well ground to obtain RHNPs (Dominic*etal.*, 2013).

2. Characterization of Si NPs:

2.1. XRD of the samples was obtained with a diffractometer (Model Lab K ShimADZU XRD-6000 powder) to determine the phases of Si NP saccording to Yalcein and Sevince(2001).

2.2. SEM /EDX: The morphology of synthesized silica was examined by SEM(Model JEOL, JSM-6400, and JAPAN)/EDX to identifying the elemental composition according to Yalcein and Sevince (2001).

2.3.TEM: The diameter and size distribution of synthesized silica were performed with TEM microscope (Model JEOL, JEM 2100 and JAPAN)according to Yalcein and Sevince (2001).

3. Laboratory and greenhouse studies:-

3.1. Fungi preparation: Rice blast fungus was isolated from infected leaves of Sakha 101 rice cultivar during 2014 season from El-Beheira governorate and identified as *P. grisea* race IE-5 according to disease reaction pattern on the international differential cultivar (Atkins *et al*., 1967).Rice brown spot fungus was isolate from rice leaf showing typical symptoms of brown spot Sakha 104 rice cultivar during 2014 season from Kafr El-Sheikh according to (Kalboush, 2007) and identified by the morphological characteristics as *B. oryzae* according to Barnett and Hunter (1972). The two fungi were cultured on PDA culture medium at 26−28^{o}C until the whole surface of the plate was covered with mycelium. Mycelia mats were gently scraped by spatula and plates were placed under wet cheese cloth for two days with continued fluorescent light to induce sporulation and finally produce spores. These spores were used as inoculum source.

3.2. Effect of different sources of silica on rice blast and brown spot diseases incidence: Experiments were carried out at RRTC, Sakha, Egypt under greenhouse conditions during 2015 seasonto study the effect of soiland spray applicationwith silica on rice seedlings. Treatments WRHA, RHNPs, white rice straw (WRSA) and RSNPsas well as K_2SiO_2 with SiO_2 content of 25% (w/v)were used as silicon sources in this study. Method of application and concentrations of supplemental silicon treatments were as follow: WRHA, RHNPs, WRSA, RSNPs and K_2SiO_2were applied to the soil prior to planting at the concentrations i.e. 0.5, 1.0 and 1.5 g per 1 kg soil.

Rice plants in their fourth leaf growth stage were sprayed 7 and 3 days before inoculationas foliar spray by the same materials of soil treatments with the concentrations i.e. 0.15, 0.30 and 0.45 g/L and two recommended fungicides Beam

(Tricyclazole 0.05/l) to blast and Del cup (Copper Sulfate Pentahydrate 0.5g/l) to brown spot were used as control**.**

3.3. Artificial inoculation: Rice plants in their fourth leaf growth stage of all soil and spray applications were sprayed with spore suspension containing (1×10^5spores/ml)each of *P. grisea* and *B. oryzae* (10 ml/pot). Non inoculated plants were sprayed with water containing the same amount of Tween 20 (0.02%), both inoculated and non-inoculated plants were kept in a moist chamber with 98−100% relative humidity for 24 hours and then transferred to greenhouse conditions. Rice seeds of Sakha 101and Hybrid 1 cultivars were used as source of plant material in this experiment. Seeds were soaked in water at around 25-30^{o}C for two days to hasten germination. Germinated seeds were planted into plastic seedling boxes (15 cm diameter). 5 g of nitrogen fertilizer was mixed with 500g of soil. Three replicates were used. Data wasrecorded10 days after planting at the fourth-leaf growth stage.

3.4. Disease assessment: Leaf blast and brown spot infection was assessed as a percentage by counting the number of infected leaves of 100randomly selected leaves per pot at 10 days after infection. The total number of type 4 lesions on the infected leaves was used as a criterion for disease severity of blast (Osman *et al*., 2002), while severity of brown spot infection was calculated as a total number of brown spot lesions per infected leaves, (Kalboush, 2007).

4. Physicochemical analysis:

4.1.SEM/EDX: The surface scan was performed using a SEM (JEOL, JSM-6400, and JAPAN) by Kim *et al*. (2002) to observethe deposition of Si in riceleaves. Before the scanning process, all samples were dried and coated with gold to enhance the electron conductivity.

4.2.TEM: Squares were excised with scissors from the top- fourth leaves. The leaf samples of rice seedling grown without silica (control) or with RHNPs applied at a rate of 1.5g/1kg soil available Si were collected and fixed immediately with 2% (v/v) glutaraldehyde and 2% (v/v) paraformaldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) at room temperature overnight and then washed with the same buffer three times for 10 min each (Kim *et al*., 2002). Afterwards, samples were post-fixed with 1% (w/v) osmium tetroxide in the same buffer at room temperature for 2h and washed twice with distilled water. The post-fixed samples were stained with 0.5% (w/v) uranyl acetate at 4^{o}C overnight. They were then dehydrated in a graded series of ethanol [30, 50, 70, 80, 95, and 100% (v/v)] and three times in 100% ethanol for 10 min each (Kim *et al*., 2002). Ultrathin sections (approximately 50 nm in thickness) were made with a diamond knife by an ultra-microtome (LKBVI). The sections were

mounted on copper grids and stained for 7 min each with 2% (w/v) uranyl acetate and Reynolds' lead citrate (Kim *et al.*, 2002). The sections were examined by TEM (JEM 2100 JOEL, JAPAN).

5. Data Analysis: Data were statistically analyzed using analysis of variance (ANOVA) of the split- plot design was applied in greenhouse experiments. The split-plot designs was adopted according to Gomez and Gomez (1984). The treatment means were compared using the least significant difference (LSD) at 5%.

RESULTS AND DISCUSSION

1. Preparation and synthesis of Si NPs:

1.1. Synthesis of RS was studied bythermal method to determinethe heating process at a constant 10 °C/min rate and under a step-wise controlledheating process. The cleaned and dried RS powders show in fig. (1A)andWRSAfig. (1B)with a slight light brown color. ExtractedSifrom RS by alkali dissolution, acid precipitation and fine pure white powders from the ash RSNPsfig. (1C).

1.2.Rice husk: The change incolor during digestion is also remarkable as the originalRH color changes from brown to light yellow on increasingthe time of digestion as displayed in fig. (1D& E). WRHAwere used for preparation of Si NPS as shown fig. (1F).

2. Characterization of SiNPs:

2.1. XRD of RSNPs showed a broad peak between 27° and 32°, centered at 30°, typical for amorphous silica fig. (2A). On the other hand, XRD of RHNPs showed a broad peak between 20° and 30°, centered at 25°, typical for amorphous silica fig. (2B). **Dominic *et al.* (2013)** reported that XRD pattern of RHNS shows a broad peak at 2· =220 which confirms the amorphous nature of RHNS.

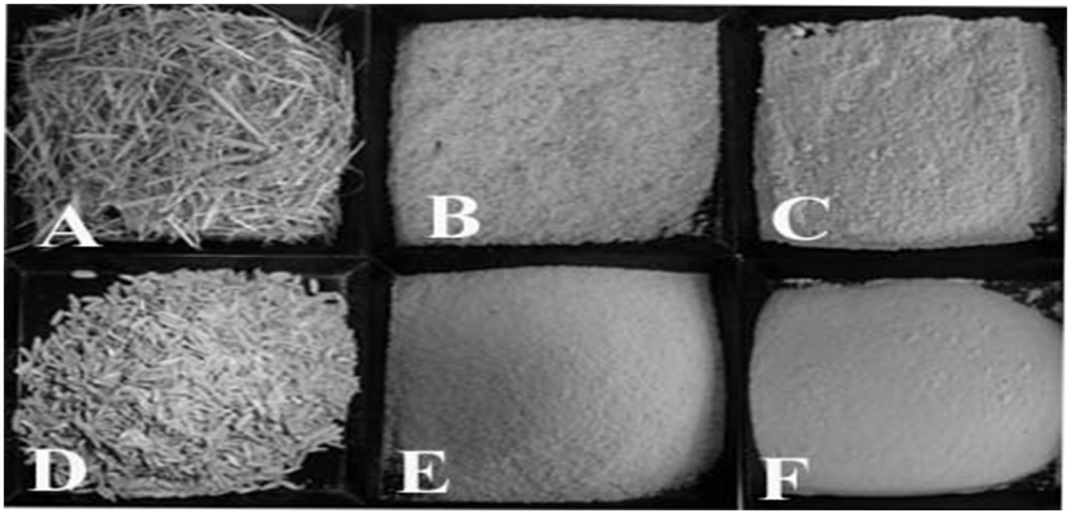

Fig. 1. Silica extraction from RS and RH, original powders of RS (A), WRS (B), RSNPs (C) RH (D), WRH (E) and RHNPs (F).

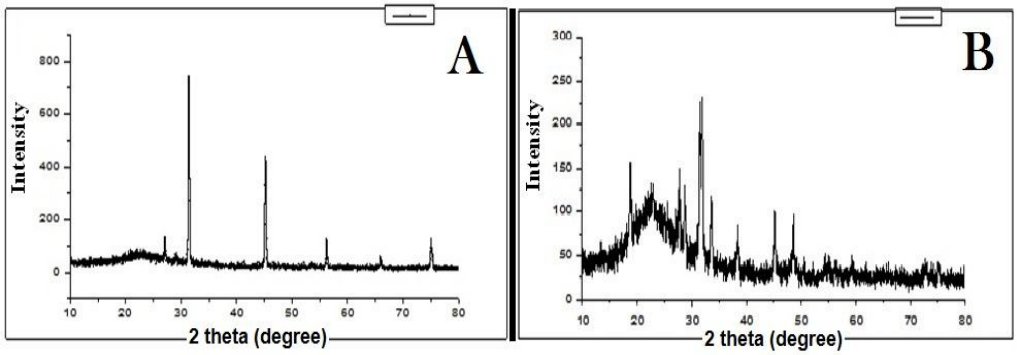

Fig. 2.XRD of synthesized RSNPs (A) and RHNPs (B).

2.2. SEM/EDX:The SEM for RSNPs and RHNPs shown in fig. (3A & C) the silica powders to be micrometer to tens of micrometer size agglomerates. Data in table (1)and fig. (3B & D)showed elemental composition of RHNPs and RSNPs. The percent of Si in RHNPs was about 22.78% while in RSNPs was 9.56%, respectively**.**

Table 1. Elemental composition of RHNPs and RSNPs.

Sample	Color	Elemental composition (Weight %)											
		Si	Na	Zn	Cu	S	Al	K	Ca	Mg	Cl	O	C
RHNPs	white	22.78	5.59	0.48	0.67	2.07	0.33	0.0	0.0	0.0	2.02	53.52	12.55
RSNPs	white	9.56	5.45	0.44	0.59	0.0	0.0	0.18	0.26	0.21	3.68	46.16	33.47

2.3. TEM images of samples dried from highly diluted RHNPs and RSNPs suspension on carbon grid surface showed the dispersed silica to be of spherical shape and size particle 73.6 nm and 133.7 nm, respectively shown in fig. (4).

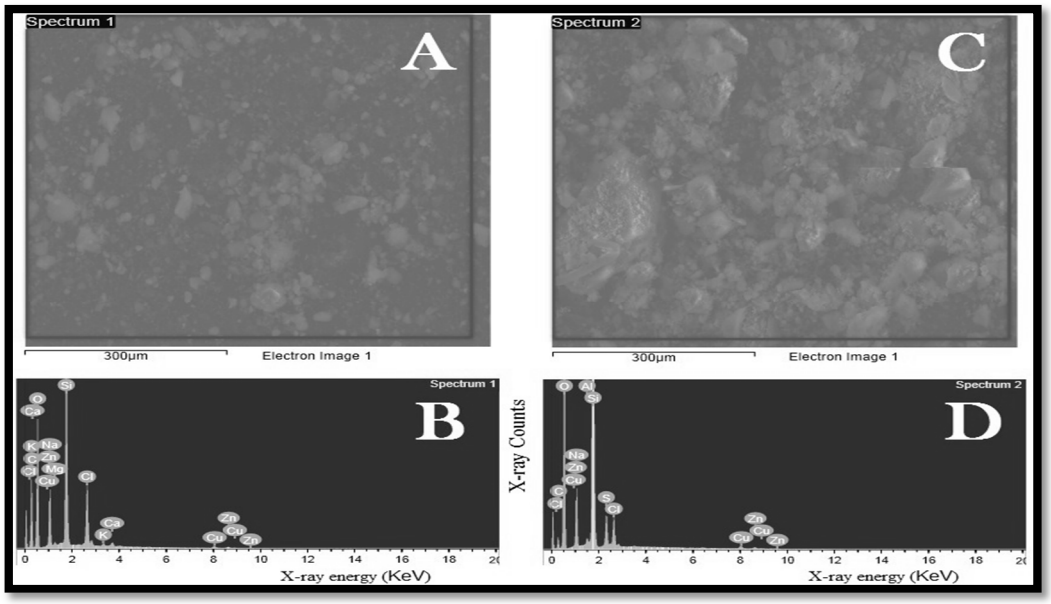

Fig.3.SEM and EDXofRSNPs (A& B) and RHNPs (C & D).

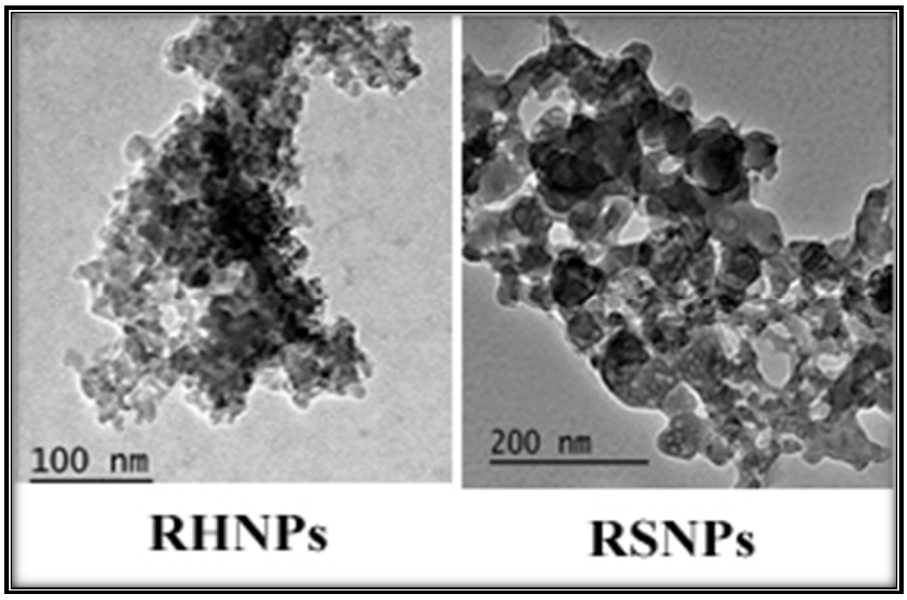

Fig.4.TEM images of RHNPs and RSNPs.

3. Laboratory and greenhouse studies:-

3.1.Effect of soil and spray application with different sources of silica NPs on controlling rice blast disease: All rates of applied silicon led to asignificantreduction inblast disease severity andincidence under artificial inoculation

with spore suspension of *P. grisea* ($1x10^5$ spores/ml) compared to non-treated plants regardless of product type table (2). Treatments with RHNPs and WRHA as soil application (1.5 g/1kg) were the most effective to reduce blast disease severity (92.56 & 90.90 %) as efficiency. Sprayed rice seedlings with different sources of Si at conc., *i.e*. 0.15, 0.30 and 0.45g/L, 7 and 3 days before artificial inoculation (BAI) with spore suspension of *P. grisea* ($1 x 10^5$spores/ml) to find out their effect against the infection by the fungus under greenhouse conditions. Data presented in table (2) show that the efficiency of silica NPs, in most cases, obviously increased by increasing the concentration. Spraying of RSNPs seven days BAI with the spore suspension gave the best effect on controlling rice blast disease 85.12% and was the highly effective in reducing the disease incidence with the conc. *i.e*. 0.45g/L.On the other hand, spraying of RHNPs and RSNPs with the conc. 0.45g/Lthree days BAI with spore suspension showed 83.88& 83.47%, respectively gave good effect in controlling rice blast disease (table 2).

Regarding cytological and pathogenic features associated with physical resistance, silicon deposited on the tissue surface decreases the number of leaf blast lesions, or increases the incubation period, as reported for the *P. grisea* and *Rhizoctoniasolani* rice pathosystems (Seebold *et al*., 2004). Moreover, Kim *et al*. (2002) reported that silicified epidermal cell walls were closely associated with the reduced severity of the blast disease *Magnaporthegrisea* in susceptible and partially resistant rice cultivars.

3.2. Effect of soil and spray application with different sources of silica NPs on controlling rice brown spot disease: Rice seedlings inoculated with spore suspension of *B. oryzae* was used. Data presented in table (3) indicated that all soil treatments with different sources of silica reduced the disease severity of rice brown spot by increasing the conc. of silica NPs. In addition, RSNPs, K_2SiO_2 followed by RHNPs 90.76, 88.46 and 86.15%, respectively were the most effective to control the brown spot disease.

Data presented in table (3) show that the efficiency of different sources of silica NPs, in most cases, obviously increased by increasing the conc. Spraying of rice seedling treated seven days before infection with WRHA, RHNPs, WRSA and RSNPs 93.07, 91.53, 88.46 and 86.15%, respectively were the most effective to control brown spot disease compared with other treatments. On the other hand, K_2SiO_2 Three days before infection gave 96.92%.

Table 2.Disease severity and incidence in rice seedlings (Sakha 101cv.) with different sources of SiNPs on rice blast disease under artificial inoculation with spore suspension of *P. grisea* (1x 10^5spore/ml) during 2015 under greenhouse conditions.

No	Method of applications	Treatments	Conc.	Disease Severity	Efficiency%	Disease incidence %
1	Soil treatments	WRHA	0.5g/1kg	180.0	77.78	53.3
2			1.0g/1kg	130.0	83.88	56.6
3			1.5g/1kg	73.30	90.90	46.6
4		RHNPs	0.5g/1kg	160.0	80.16	53.3
5			1.0g/1kg	150.0	81.40	50.0
6			1.5g/1kg	60.00	92.56	26.6
7		WRSA	0.5g/1kg	270.0	66.52	66.6
8			1.0g/1kg	180.0	77.68	63.3
9			1.5g/1kg	150.0	81.40	60.0
10		RSNPs	0.5g/1kg	183.0	77.33	63.3
11			1.0g/1kg	163.3	79.75	56.6
12			1.5g/1kg	153.3	80.99	53.3
13		K_2SiO_2	0.5g/1kg	220.0	72.27	66.6
14			1.0g/1kg	123.3	84.71	56.6
15			1.5g/1kg	110.0	86.40	43.3
16	7 day spray before inoculation	WRHA	0.15g/L	226.6	71.90	80.0
17			0.30g/L	183.3	77.30	76.6
18			0.45g/L	163.3	79.75	66.6
19		RHNPs	0.15g/L	263.3	67.35	80.0
20			0.30g/L	206.6	73.34	73.3
21			0.45g/L	183.3	77.30	70.0
22		WRSA	0.15g/L	260.0	67.76	80.0
23			0.30g/L	243.3	69.84	73.3
24			0.45g/L	186.6	76.86	73.3
25		RSNPs	0.15g/L	196.6	75.62	80.0
26			0.30g/L	166.6	79.35	73.3
27			0.45g/L	120.0	85.12	66.6
27		K_2SiO_2	0.15g/L	366.6	54.55	80.0
29			0.30g/L	246.6	69.42	80.0
30			0.45g/L	140.0	82.64	80.0
31		Beam	0. 5g/L	3.340	99.58	3.34
32	3 day spray before inoculation	WRHA	0.15g/L	210.0	73.96	80.0
33			0.30g/L	206.0	74.00	80.0
34			0.45g/L	166.6	79.35	70.0
35		RHNPs	0.15g/L	160.0	80.16	83.3
36			0.30g/L	160.0	80.16	80.0
37			0.45g/L	130.0	83.88	60.0
38		WRSA	0.15g/L	206.0	74.00	80.0
39			0.30g/L	193.3	76.00	76.6
40			0.45g/L	180.0	77.68	70.0
41		RSNPs	0.15g/L	180.0	77.68	80.0
42			0.30g/L	180.0	77.68	76.6
43			0.45g/L	133.3	83.47	56.6
44		K_2SiO_2	0.15g/L	210.0	73.96	70.0
45			0.30g/L	186.6	76.86	70.0
46			0.45g/L	116.6	85.54	53.3
47		Beam	0.5g/L	3.340	99.58	3.34
48	Control		-	806.6	-	100
LSD 5%			-	19.67	-	9.10

Table 3.Disease severity and incidence in rice seedlings (Hybrid 1) with different sources of SiNPs on brown spot disease under artificial inoculation with spore suspension of *B. oryzae* (1x 10^5spore/ml) during 2015 under greenhouse conditions.

No	Method of applications	Treatments	Conc.	Disease Severity	Efficiency %	Disease incidence %
1	Soil treatments	WRHA	0.5g/1kg	186.00	57.00	53.34
2			1.0g/1kg	116.67	73.07	36.34
3			1.5g/1kg	76.670	82.80	33.34
4		RHNPs	0.5g/1kg	210.00	51.53	56.67
5			1.0g/1kg	86.670	79.99	36.67
6			1.5g/1kg	60.000	86.15	26.67
7		WRSA	0.5g/1kg	163.34	62.30	60.00
8			1.0g/1kg	136.67	68.46	40.00
9			1.5g/1kg	105.00	75.78	26.67
10		RSNPs	0.5g/1kg	90.000	79.23	53.34
11			1.0g/1kg	60.000	86.15	26.67
12			1.5g/1kg	40.000	90.76	26.67
13		K_2SiO_2	0.5g/1kg	170.00	60.76	66.67
14			1.0g/1kg	50.000	88.46	63.34
15			1.5g/1kg	50.000	88.46	40.00
16	7 day spray before inoculation	WRHA	0.15g/L	106.67	77.69	53.34
17			0.30g/L	86.670	80.00	30.00
18			0.45g/L	30.000	93.07	26.67
19		RHNPs	0.15g/L	53.340	86.69	30.00
20			0.30g/L	43.340	89.90	26.67
21			0.45g/L	36.670	91.53	16.67
22		WRSA	0.15g/L	153.34	64.61	56.67
23			0.30g/L	86.670	80.00	43.34
24			0.45g/L	50.000	88.46	36.67
25		RSNPs	0.15g/L	163.34	62.32	70.00
26			0.30g/L	123.34	71.53	53.34
27			0.45g/L	60.000	86.15	30.00
27		K_2SiO_2	0.15g/L	153.34	64.61	90.00
29			0.30g/L	100.00	76.92	86.67
30			0.45g/L	76.670	82.30	66.67
31		Del-Cup	1ml/L	86.670	80.00	60.00
32	3 day spray before inoculation	WRHA	0.15g/L	193.34	55.38	56.67
33			0.30g/L	126.67	73.76	46.67
34			0.45g/L	83.340	80.67	33.34
35		RHNPs	0.15g/L	80.000	81.53	50.00
36			0.30g/L	80.000	81.53	43.34
37			0.45g/L	46.670	89.23	23.34
38		WRSA	0.15g/L	220.00	49.23	73.34
39			0.30g/L	173.34	60.00	66.67
40			0.45g/L	170.00	60.76	36.67
41		RSNPs	0.15g/L	270.00	37.69	86.67
42			0.30g/L	223.34	48.50	80.00
43			0.45g/L	76.670	82.30	66.67
44		K_2SiO_2	0.15g/L	123.34	71.53	50.00
45			0.30g/L	65.000	85.00	43.34
46			0.45g/L	13.340	96.92	10.00
47		Del-Cup	1ml/L	23.340	94.61	20.00
48	Control		-	433.34	-	100.0
LSD 5%			-	13.30	-	2.62

Under silicon deficient conditions, some diseases such as blast, brown spot and sheath blight can be extremely threatening to rice cultivation (Rodrigues &Datnoff, 2005). Accumulated silicon in rice tissues enhances resistance against insects and diseases, increases erectness of leaves resulting in increased photosynthesis, improves water usage, and reduces toxicity of heavy metals and cuticular transpiration (Epstein, 1994). It has also been reported that rice blast severity is directly related with silicon deficiency in soils (Kim *et al*., 2002).

4. Histo-chemicalresult:

4.1. Localization and quantification of Si deposition in rice leaves by SEM/EDX: SEM/EDX observationsand X-ray spectra of adaxial surfaces of the fourth rice leaves in soil treatment with 1.5g/kg show different types of silicified cellssuch as increase intrichome cell size of dumbbell-shaped or ladder-like silica cellsand small scattered silica cells **(fig. 5).**

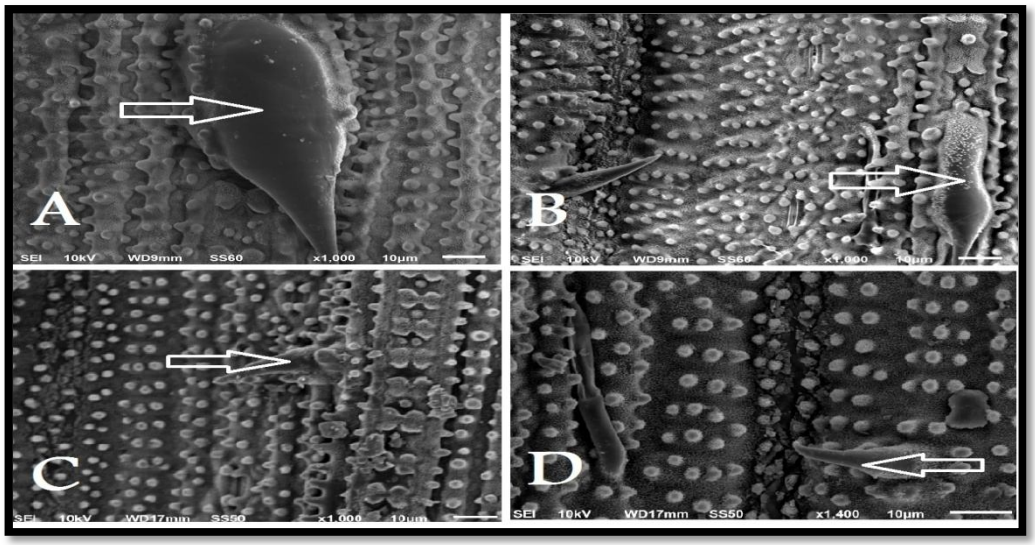

Fig.5.SEM (1000 x magnification) of adaxial surface of rice leaf epidermis with different kinds of silica cells observed: Size of Trichome cell in soil treated with RHNPs(A&B), small scattered cells in non-treated plant (C&D). Bar, 10 µm.

The corresponding EDX spectra compared with the SEM images demonstrated difference in silicon content between siliconsoil treated by RHNPs 13.75% and non-treated plants 10.6%. Plants treated by RHNPs contained more silicon in comparison with non-treated ones.On the other hand, plants treated with silicate show thatsodium was absent but in control plants sodiumwas detected in **fig. (6)**.

Hayasaka*et al*. (2008)reported the Si-enhanced density of silicon layer in the leafepidermis acts as a physical barrieragainst fungus penetration. Farnaz*et al*.**,** 2012 indicated that SEM/EDX analysisof silica application led to a significant increase in

concentrationof accumulated silicon in silica cells, especially dumbbellshapedones which are believed to affect the mechanicalpropertiesof the rice leaf epidermis, and consequently increased their resistance to rice blast incidence.

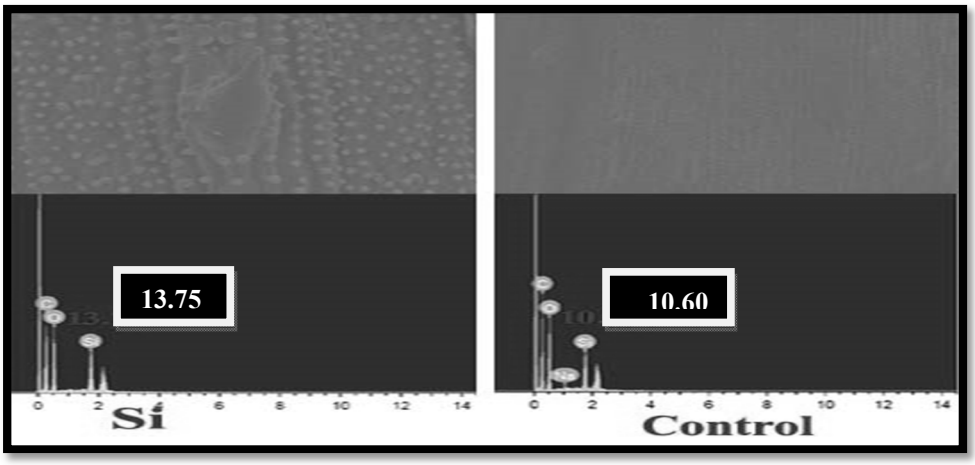

Fig. 6. A Scanning electron microscopy and X-ray spectra of soil treated by RHNPs (1.5g/1kg) Sakha101 rice leaves at the fourth rice leaves. Numbers in the micrograph indicate the silica content (13.75 and 10.6 %) on leaves surface area treated with RHNPsand non-treated leaves respectively for X-ray emission.

4.2: TEManalysisof silica cell ultrathin sections of leaf samples were observed by TEM. The Si layers were observed in Si-treated epidermal cell walls, cuticle and the thickness of the silicon layer was seen to be increased by soil RHNPs treated leaves (Fig. 7).Electron-dense silicon layers were frequently found beneath the cuticle in epidermal cell walls of silicon-treated plants. Increasing levels of silicon were detected in the outer regions of epidermal cell walls. Silicon was present mainly in epidermal cell wallsand intercellular spaces within sub-epidermal tissues.In contrast, the chloroplast structure of mesophyll cells of Si-treated rice leaves was relatively intact some starch grains visible compared with non-treated plants (Fig. 7). Plants absorb silicon in the form of mono silisic acid $Si(OH)_4$, which is accumulated in cell walls as silica gel. SiO_2-H_2O is also referred to as 'Opals' or 'Phytolits' in leaves (Rodrigues and Datnoff, 2005). The highest rate of silicon accumulation in leaves can be observed in epidermal cells (Rodrigues and Datnoff, 2005).

Obtained silica extracted from grain husk and rice straw good material to manage rice blast and brown spot diseases. Use of rice straw and husk in the production of silica under control to preserve the environment of the combustion processes and good substitute for fungicides. the accumulation of silicon in different

areas of tissue rice leaves may play a role in increasing plants resistant to rice blast and brown spot diseases.

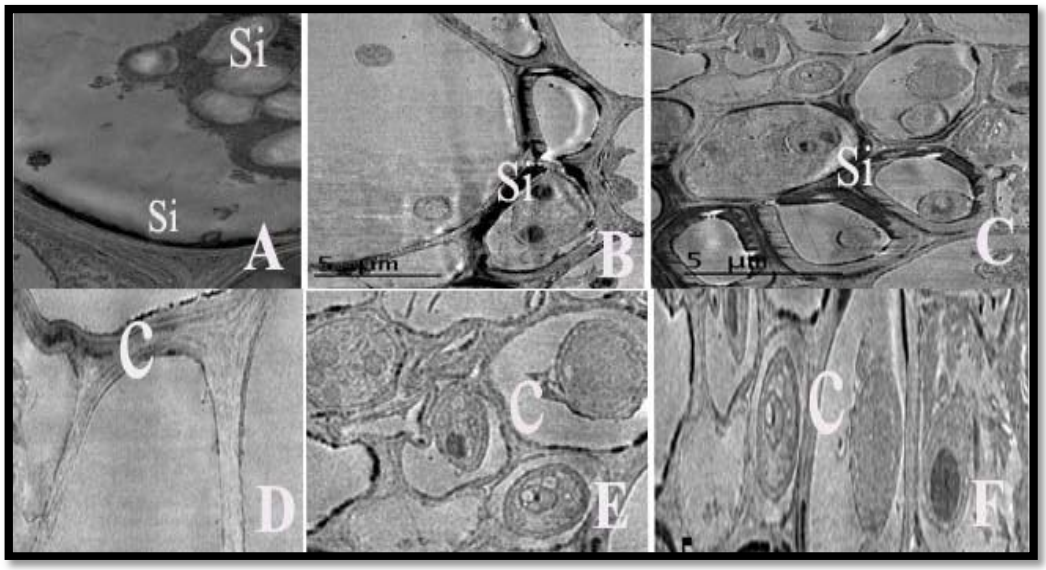

Fig.7.Transmission electron microscopicof cell wall from rice leaves; A, B and C: Leaf epidermis of a silicon treated plant with RHNPs applied at a rate of1.5g/1kg soilplant-available Si.D, E and F: Leaf epidermis of a control plant grown without silicon fertilizer.

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تصنيع وتوصيف النانو سيليكا الطبيعية والنشاط المضاد ضد اثنان من فطريات الأرز الممرضه

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تم استخلاص السيليكا من قشرالحبوب وقش الأرز بطرق مختلفة، وصفت باستخدام الأشعة السينية، الماسح الاليكتروني والناقل الاليكتروني. باستخدام الاشعة السينية تم قياس حجم البلورات لهذه الجزيئات عن طريق معادلة شيرر. مقاس حجم جزيئات السيليكا المنتجة من قشر الحبوب وقش الأرز ٧٣,٦ نانوميتر و ١٣٣,٧ نانوميتر علي التوالي. وكانتنسبة جزيئات النانو سيليكا المنتجة من قشر وقش الأرز كانت ٢٢,٧٨ و ٩,٥٦ % علي التوالي.

استخدمت جزيئات السيليكا المتناهية الصغر في مقاومة مرضى لفحة الأرز والتبقع البني ذات فعالية وذلك تحت ظروف العدوي الصناعية بالصوبة الزجاجية في موسم ٢٠١٥.حيث كانتمعاملة التربة بمعدل ١,٥ جم /كجم تربة لكل من السيليكا المنتجة منقشرالارز المتناهية الصغر وقشر الأرز البيضاءبعد الحرق كانت أفضل المعاملات في تقليل شدة الإصابة بمرض اللفحة مقارنة بالمعاملات الأخرى وايضا بدون معاملة حيث كانت الفعالية ٩٢,٥٦، ٩٠,٩% علي التوالي.من ناحية اخري،ادي رش نباتات الارز بسيليكات البوتاسيوم، بقشر الارزالبيضاء بعد الحرق و قشرالارز المتناهية الصغر الي فعالية (٩٦,٩٢، ٩٣,٠٧و ٩١,٥٣% علي التوالي) كان اكثر فعالية في مقاومة مرضالتبقع البني مقارنة بالمعاملات الأخرى .

أيضا تلاحظ من استخدام الماسح الاليكتروني و EDX و مع الاشعة السينية لسطح الورقة الرابعة لنبات الأرزكمعاملة تربة بمعدل ١,٥جم/كجم اعطت انواع مختلفة من الخلايا المسلكنة.وجد أيضا من خلال EDX مقارنة ب SEMان في محتوي الأوراق من السيلكون نتيجة المعاملة بجزيئات النانو المنتجة من قشر حبوب الأرز كانت ١٣,٧٥ % والغير معاملة كانت ١٠,٦ %. وجد ان تراكم السيليكا في الاوراق المعاملة بقشر حبوب الارزكمعاملة تربة بمعدل ١,٥جم/كجم، زادت طبقات السيليكا في جدران الخلايا البشرة، وسمكها. أيضا، المناطق الخارجية من جدران الخلايا البشرة والمساحات بين الخلايا داخل أنسجة شبه الأدمي.ووجد ان طبقات السيليكون لها دوهام في مقاومة نباتات الأرز والسيطرة على الأمراض الأرز تحت الدراسة.