Nanoparticles Constructed from *Neosartorya hiratsukae* to Control *Drechslera oryzae* Causing Brown Spot of Rice

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ABSTRACT

The toxic chemical fungicides for plant disease control has been used by farmers for years. Those are faced hazardous effect to the surrounding environment, and the chemicals were toxic chemical residue remained in rice products which harmful to the consumers. It is increasing interested to search the new biological control agent to control the pathogen and to be promising as a biocontrol agent against the rice bast incidence in seriously infested rice fields. The aims of project was proved the fungus *Neosartorya hiratsukae* as a biological control agent to brown spot of rice (*Oryza sativa*) caused by *Drechslera oryzae* and developed a novel nanoparticles constructed from *N. hiratsukae* to control the rice brown spot. Result revealed that the aggressive isolate of *D. oryzae* was significantly inhibited by *N. hiratsukae* antagonistic fungus in bi-culture antagonistic evaluation. The growth of *D. oryzae* in bi-culture test was inhibited fungal colony and sporulation by 51 and 55 %, respectively. Bioassay of crude metabolite extract by methanol from *N. hiratsukae* gave the good inhibition of the colony growth and sporulation of *D. oryzae* which the ED_{50} of 1.7 and 92 ppm., respectively. The nanoparticles constructed from *N. hiratsukae* using electron spinning method found that nanoparticles SH-M expressed the best inhibition of the pathogen growth both colony and sporulation of the tested rice pathogen with the ED_{50} of 4 ppm. It is observed that nanoparticles SH-M had more effective control the pathogen than crude metabolite extract from *N. hiratsukae*. Pot experiment showed that nanoparticles SH-M actively expressed antifungal activity against brown spot of rice more than the inoculated control. It is interested that the rice plants treated with nanoparticles SH-M resulted to be better plant stands than the inoculated control. This is recorded for the first time using new antagonist, *N. hiratsukae* and its active compounds both in crude extracts and nanoparticles to control brown spot of rice.

Keywords: Neosartorya hiratsukae, rice, brown spot, rice, nanotechnology

1. INTRODUCTION

The major food crops in Asia is rice (*Oryza sativa* L.) It is the most widely consumed staple food of the world population in Asia and the West Indies. It is the third-highest production in the world FAO. [1] *Drechslera oryzae* (Breda de Haan) Subram. & Jain causing rice brown leaf spot become epidemic disease in Thailand and Cambodia. Brown leaf spot of rice can infect the stems in seedlings and mature plants. The symptom showed blight in brown colour on leaves, and seedlings where grown from infected seeds [2]. It causes brown spot of rice leaves especially in seedling stage. It spreads to the rice fields which causing seedlings death until 58% [3, 4] Chemical fungicides are applied to control the disease by the rice growers and the repeatedly application of chemical fungicides leading development of pathogen resistant to those chemicals. The antagonistic fungi against plant pathogens has been widely searched to control the diseases. Tathan *et al.* [5] stated that antagonistic fungi *Chaetomium* spp., *Chaetomium cochliodes*, *Chaetomium cupreum*, *Chaetomium brasilense*, *Chaetomium elatum* and *Chaetomium globosum* effectively supressed leaf spot pathogen of rice caused by *D. oryzae*. These *Chaetomium* spp. are reported to produce bioactive metabolites to inhibit the sporulation of *D. oryzae.* Recently, *Neosartorya hiratsukae* used in this experiment is reported to produce chevalone G and aszonapyrone C, 7-chlorofischerindoline and brasiliamide H that inhibited *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Salmonella enterica* serovar typhimurium. Moreover, *N. hiratsukae* also reported tom produce 7-chlorofischerindoline that

expressed cytotoxicity to HeLa, HepG2, HT-29, KB, MCF-7, and Vero cell lines with the IC50 ranged from 45 to ppm 63. [6]. Many researchers found this antagonistic fungi, *Neosartorya fischeri*, *Neosartorya glabra*, *Neosartorya hiratsukae*, *Neosartorya takakii*, and *Neosartorya tatenoi* against chilli anthracnose caused by *Colletotrichum capsici* [7, 8] *N. hiratsukae*, *Neosartorya pseudofischeri*, *Neosartorya aureola*, *Neosartorya spinosa*, and *Neosartorya fennelliae* also proved to control leaf spot of kale caused by *Alternaria brassicicola* [9]. Moreover, *N. hiratsuka* EU06 actively inhibited *Phomosis asparagi* causing asparagus anthracnose over 59 %. The objective of reserch project was evaluated the nanoparticles constructed from *N. hiratsukae* strain MST to control *D. oryzae* causing brown leaf spot of rice.

2. MATERIALS AND METHODS

2.1. Isolation of Drechslera oryzae causing brown leaf spot of rice (Oryza sativa)

The sample of brown leaf spot symptom was isolated by tissue transplanting method. The advanced margin from disease lesion was placed on water agar medium and maintained at room temperature (28-30 C). The hyphal tips were moved to potato dextrose agar (PDA) to receive pure culture.

2.2. Antagonistic fungus, Neosartorya hiratsukae strain MST

The antagonistic fungus, *Neosartorya hiratsukae* MST is obtained from Biological control Research Unit, Agricultural Technology Faculty, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand.

2.3. Pathogenicity test

The isolated pathogen was proved to be pathogenic isolate according to Koch's Postulate.by detached leaf method The agar plugs of fungal pathogen were inoculated onto the wounded leaves of rice var KorKor1. The agar plugs without fungal pathogen were transferred onto wounded leaves served as controls. The experiment was set up as Completely Randomized Design (CRD) with four replications. The inoculated and non-inoculated pathogen on the wounded leaves were incubated in moist chambers at room temperature of 27-30 C for 7-10 days until the lesions appeared. The infected leaves were re-isolated to get the pure isolate of the same pathogen. Disease index was scored as 0 = no symptom and 10 = 76-100 % leaf blight [10].

2.4. Bi-culture test between Neosartorya hiratsukae MST and Drechslera oryzae

N. hiratsukae was evaluated to inhibit *D. oryzae* causing brown leaf spot of rice var Korkor1. Completely

Randomized Design (CRD) was arranged with 4 repeated experiments. The tested antagonists was separately cultured on potato dextrose agar (PDA) at room temperature (30ºC) for 15 days. The antagonistic agar plugs of 0.5 cm were cut from actively growing edge of cultures, then transferred to PDA plates at one side and the agar plugs (0.5 cm) of tested pathogen was transferred at opposite side in bi-culture plates. The agar plugs of *N. hiratsukae* and *D. oryzae* were separately moved to PDA plates which served as the controls. Bi-culture plates were incubated at room temperature (30 º C) for 20 days. Colony diameter (cm) and sporulation in bi-culture plates and control plates were measured using haemacytometer. The inhibition of colony growth and sporulation of *D. oryzae* were calculated by the following formula: % inhibition = (A-B) / A $\times$100, where, A is colony diameter or sporulation in bi-culture and control plates, and B represented the colony diameter or sporulation in the bi-culture plate. Data were calculated analysis of variance (ANOVA) and means were compared using Duncan Multiple's Range Test (DMRT) at P = 0.05.

2.5. Crude extracts of Neosartorya hiratsukae MST testing against Drechslera oryzae

The crude extracts of *N. hiratsukae* was tested for inhibition of *D. oryzae*. The experiment was designed in 3$\times$6 factorial in Completely Randomized Design (CRD) with repeated experiments. The crude hexane, ethyl acetate and methanol extracts were in combination with the concentrations of 0, 10, 50, 100, 500 and 1,000 ppm. Each crude extract was dissolved 2% dimethyl sulfoxide, mixed into PDA, then autoclaved at 121°C, 15 lbs for 30 mins. The pathogenic isolate of *D. oryzae* was transferred its agar plug to the middle of PDA incorporated with the extract. The tested plates were incubated at room temperature and observed until *D. oryzae* in the control plates grew in full plate. Data were gathered spore number and colony diameter (cm), then statistically computed ANOVA and mean comparison was calculated using DMRT) at P = 0.05. The probit analysis program was computed the effective dose (ED$_{50}$).

2.6. Natural products nano-particles from Neosartorya hiratsukae MST testing against Drechslera oryzae

The nano-particles constructed from *Neosartorya hiratsukae* (nano NH-H, nano-NH-E, nano-NH-M) were offered from Biological control Research Unit, Faculty of Agricultural Technology, KMITL, Bangkok, Thailand. Two factors factorial experiment in CRD was performed with four repeated experiments. Nano NH-H, nano-NH-E, nano-NH-M were tested in combination with the concentrations of 0, 1, 3, 5, 7 and 10 ppm. The method was followed the above experiment.

2.7. Pot experiment testing for efficacy of nanoparticles from Neosartorya hiratsukae MST to control brown leaf spot of rice

Randomized completely block design (RCBD) was performed with four repeated experiments. Treatments were set up as follows: T1= inoculated control, T2= nano-NH-M derived from *N. hiratsukae* MST at the concentration of 10 ppm, and T3= carbendazim fungicide (1 g/1L). 15 seedlings of rice var Korkor1 were plants in pots containing sterilized soil in each experimental unit. The tested pathogen, *D. oryzae* was cultured on PDA for 15 days before collecting the conidia to make inoculate $1x10^5$ spores/ml. All tested plants were inoculated by spraying onto the wounded lesion on leaves. The inoculated plants were sprayed either nano-NH-M or carbendazim fungicide, and maintain in greenhouse for one month. The plant height (cm), fresh and dried weight of plants (g). were recorded   Disease index was scored as 1= no symptom, 2= 1-25 %, 3= 26-50%, 52-75v%, 76-100 %.

3. RESULTS AND DISCUSSION

3.1. Isolation of Drechslera oryzae causing brown leaf spot of rice (Oryza sativa)

Drechslera oryzae was isolated and identified to be causal agent of brown leaf spot. It was proved to be aggressive isolate to cause the disease according to Koch's postulate. *D. oryzae* belongs to Pleosporaceae, Kingdom Fungi. The fungus grew on PDA for 19 days with brown black in color of colony. It produces conidia on conidiophore either in the top or side of conidiophore, conidia 3-9 septa (Fig. 1). With this, brown leaf spot of rice is reported to infect rice either in seedlings or mature plants [2].

3.2. Antagonistic fungus, Neosartorya hiratsukae strain MST

The fungus belongs to Eurotiaceae, Ascomycotina, Kingdom Fungi. Colony is white color in PDA at 30 days. It produces perithecia which contain many asci which rounded shape or ovate, 8-ascospore/ascus and ascospores have ridges along the ascopores (Fig.2).

3.3. Pathogenicity test

The isolated *Drechslera oryzae* was proved to be pathogenic to rice var Korkor2 causing brown leaf spot. The inoculated pathogen had high disease index of level 5. The non-inoculated control had no infection (Fig. 3). *D. oryzae* is reported to cause brown leaf spot of rice in seedling stage [4].

3.4. Bi-culture test between Neosartorya hiratsukae strain MST and Drechslera oryzae

Bi-culture test showed *N. hiratsukae* strain MST actively inhibited the tested pathogen, *D. oryzae* at 52 % and inhibited spore production of 56 % in 10 days. It observed that *N. hiratsukae* grown over the colony of *D. oryzae* causing brown spot of rice var Korkor1. Result was similar to report that *Neosartorya* species could be controlled other phytopathogens which inhibited *Colletotrichum coffeanum* causing coffee antracnose [11] and *Neosartorya aureola*, *Neosartorya fenneliae*, *Neosartorya hiratsukae*, *Neosartorya pseudofischeri*, *Neosartorya spinosa* actively suppressed *Colletotrichum musae* causing banana anthracnose in dual culture tests [12]. Moreover, *Phomopsis asparagi* causing asparagus stem blight was reported to *Neosatorya* spp. [9] and *Alternaria brassicicola* causing leaf spot of kales was inhibited by *N. aureola, N. fennelliae N. hiratsukae, N. pseudofischeri* and *N. spinosa*, [8].

3.5. Crude extracts of Neosartorya hiratsukae strain MST testing against Drechslera oryzae

The biomass of *Neosartorya hiratsukae* strain MST were extracted to yield crude hexane, crude, crude ethyl acetate and crude methanol (Fig.5). Results showed that crude hexane extracted from *N. hiratsukae* at 10, 50, 100, 500 and 1,000 ppm eliminated the sporulation of *D. oryzae* at 43, 38, 34, 36 and 48 %, respectively. Crude ethyl acetate extract at 10, 50, 100, 500 and 1,000 ppm eliminated sporulation of 14, 9, 40, 50 and 55v %, respectively. Crude methanol gave the highest spore inhibition at 10, 50, 100, 500 and 1,000 ppm, 66, 91, 95, 96, 97 %, respectively (Table 1, Fig.6). Similar result stated that nanoparticles derived from *Chaetomium brasiliense* namely nano-CBM at 10 ppm inhibited *D. oryzae* causing brown spot of rice [13]. With this, The other antagonistic fungi, *Chaetomium brasilense*, ,*Chaetomium cochliodes*, *Chaetomium cupreum, Chaetomium elatum* and *Chaetomium globosum* were reported to antagonize *D. oryzae* causing brown leaf spot of rice [5]. The research findings were in related with reports on crude extracts of *Neosartorya* spp. used to control *C. coffeanum* (coffee anthracnose) [11]. Fungal metabolites from *Neosartorya aureola, Neosartorya fenneliae*, *Neosartorya hiratsukae*, *Neosartorya pseudofischeri* and *Neosartorya spinosa* and inhibited *C. musae* causing wilt of banana [12]. Crude extracts derived from *N. hiratsukae*, *N. pseudofischeri*, *N. aureola*, *N. spinosa*, *N. fennelliae* reported to inhibit *A. brassicicola* (leaf spot of kales) [8]. Moreover, the active metabolites extracted from *Neosartorya* spp. were reported to inhibit *Phomopsis asparagi* (asparagus stem blight) [9]. It concluded that methanol crude extract gave the best result to inhibit *D. oryzae* which the ED_{50} value of 1.5 ppm, and followed by crude ethyl acetate extract which the ED_{50} value of 544 ppm.

3.6. Natural products nano-particles from Neosartorya hiratsukae testing against Drechslera oryzae

Crude hexane, crude ethyl acetate and crude methanol derived from *N. hiratsukae* strain MST were constructed by electron spinning technique to yield nanoparticles, nano-NHH, nano-NHE and nano-NHM, respectively according to the method of Dar et al. [14] The characterics of nanoparticles contrasted from *N. hiratsukae* strain MST can be seen in Fig. 7. The constructed nano particles from crude extracts of *N. hiratsukae* showed that nano-NHH inhibited the colony growth of *D. oryzae* at concentrations of 1, 3, 5, 7 and 10 ppm which were 28, 31, 40, 49 and 54 %, respectively which the ED_{50} value of 7.7 ppm (Table 2). Nano-NHE inhibited the tested pathogen at 1, 3, 5, 7 and 10 ppm. Which were 40, 41, 41, 55 and 60 %, respectively which the ED_{50} value of 5.28 ppm Nano-NHM inhibited the colony growth at 1, 3, 5, 7 and 10 ppm which were 40, 40, 44, 54 and 64 %, respectively which the ED_{50} value of 4.73 ppm. Spore production of *D. oryzae* causing brown leaf spot of rice var Korkor1 was inhibited by nanoparticles derived from *N. hiratsukae*. NHH at concentrations of 1, 3 Nano, 5, 7 and 10 ppm. inhibited spore production of *D. oryzae* of 15, 27, 33, 68 and 88 %, respectively. Spore inhibition of the tested pathogen was shown in nano-NHE at concentrations of 1, 3, 5, 7 and 10 ppm which were 20, 20, 44, 70 and 93 %, respectively. Nano-NHM showed that the production of pathogenic spores was increased from 1 to 10 ppm, concentrations at 1, 3, 5, 7 and 10 ppm inhibited spore production of 15, 16, 39, 65 and 94 %, respectively (Table 3). The ED_{50} values of nano-NHH, nano-NHE and nano-NHM to inhibit *D. oryzae* were 4.6, 4.11 and 4.63 ppm, respectively. *D. oryzae* causing brown leaf spot of rice was inhibited by crude hexane, ethyl acetate and methanol extracted from *Chaetomium cochliodes* which value of ED_{50} value was 66.45 ppm. [15].

The agricultural nanotechnology is started to be a new tool for plant disease management by restructuring natural active metabolites at the molecular level. The nanomaterials contain bioactive compounds rapidly and effectively penetrated through plant tissues and cells and help to increase the stability of active compounds to control plant disease. It was reported that natural product of nano-particles derived from *Ch. cochliodes* CTh05 actively inhibited *Magnaporthe oryzae* causing rice blast for the first time [16]. Moreover, there were reported that nano-CCoM at 7 µg/mL, followed by nanoCCoE and nanoCCoH derived from *Chaetomium cochliodes* isolate CTh05 could be reduced the blast infection in 30 days. Further report on antifungal efficacy of microbial nano-particles constructed from *Chaetomium elatum*, against rice blast pathogen in rice var. PSL 2 in Thailand was stated that nano-CEE, nano-CEM and nano-CEH inhibited sporulation of *M. oryzae* which the ED50 values of 7.89, 8.66 and 16.7 µg/mL. respectively [17].

3.7. Pot experiment testing for efficacy of nano-NHM from Neosartorya hiratsukae to control Drechslera oryzae

Nano-NHM derived from *N. hiratsukae* MST at the concentration of 10 ppm expressed the growth of *D. oryzae* (brown leaf spot of rice) in greenhouse after treated every 7 days for 4 weeks. DI inT1 (inoculated control with *D. oryzae*) was 4.5, 3.5 and 6.0 at 1, 2 and 4 weeks, respectively. T2 (Nano-NHM) showed DI 4.75, 4.5 and 5.0 while T3 (carbendazim) showed DI at 4.5, 4.5 and 4.00 after 1, 2 and 4 weeks, respectively (Table 1). The infected rice seedlings var Korkor1 was not significantly decreased in T2 and T3 when comparted with inoculated control with *D. oryzae* (Table4). Many researchers reported that living spores of *N. fischeri*, *N. glabra*, *N. hiratsukae*, *N. takakii* and *N. tatenoi* gave a good control chilli anthracnose caused by *Colletotrichum capsici* [7]. Results showed that nano-NHM derived from *N. hiratsukae* gave significantly higher number of tillers, plant height and roots, fresh weight of plant and dry weight of plants and roots which were 16, tillers 102 cm, 37 g, 5 g and 1.5 g, respectively, than the carbendazim treatment which were 10 tillers, 78 g, 22 g, 2.8 g and 0.73 g., respectively (Table %). Nano-NHM showed a potent to promote plant growth of rice var Korkor1 in greenhouse trial which significantly better the carbendazim chemical fungicide. Interestingly, similar research finding found that the tested nano-particles from *Chaetomium* spp. caused pathogenicity lost of rice blast pathogen. Moreover, nano-CBH from *Chaetomium brasiliense* treated to rice leaves proved to produce Sakuranertin and Oryzalexin B as phytoalexin against blast disease [17].
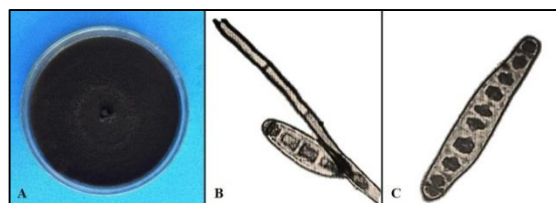

Figure 1. *Drechslera oryzae* ;A= colony on PDA, B= conidium and conidiophre, C= conidium
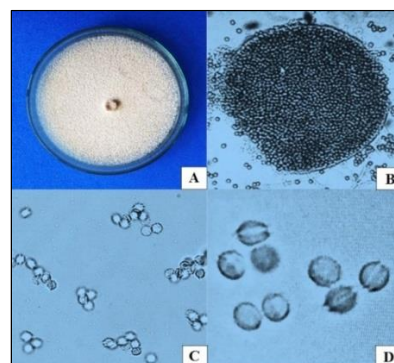

Figure 2. *Neosartorya hiratsukae* ; A= colony on potato dextrose agar at 30 days, B= ascocarp and asci, C = ascospores , D= ascospores.

Figure 3. Pathogenicity test A= non-inoculated with *Drechslera oryzae*, B= non-inoculated with *Drechslera oryzae*

Figure 4. Bi-culture test between *Neosartorya hiratsukae* and *Drechslera oryzae*
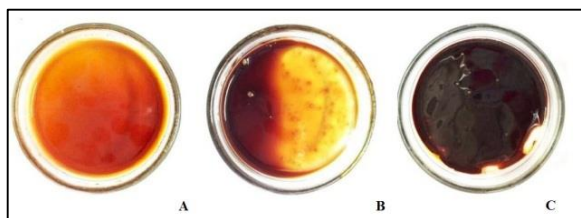

Figure 5. Crude extracts of *Neosartorya hiratsukae* strain MST, A= crude hexane, B= crude, ethyl acetate, C= crude methanol

Figure 6. Crude methanol extract of *Neosartorya hiratsukae* against *Drechslera oryzae*

Figure 7. Nanoparticles of *Neosartorya hiratsukae*; A= nano-NHH, B= nano-NHE and E= nano-NHM

Figure 8. Nanoparticles constructed from *Neosartorya hiratsukae* strain MST against *Drechslera oryzae* ; A= nano **-**NHH, B= nano-NHE and E= nano-NHM

Figure 9. Pot experiment testing for efficacy of nano NHM from *Neosartorya hiratsukae* to control brown leaf spot of rice

Table 1. Inhibition of *Drechslera oryzae* treated with crude extract of *Neosartorya hiratsukae* strain MST

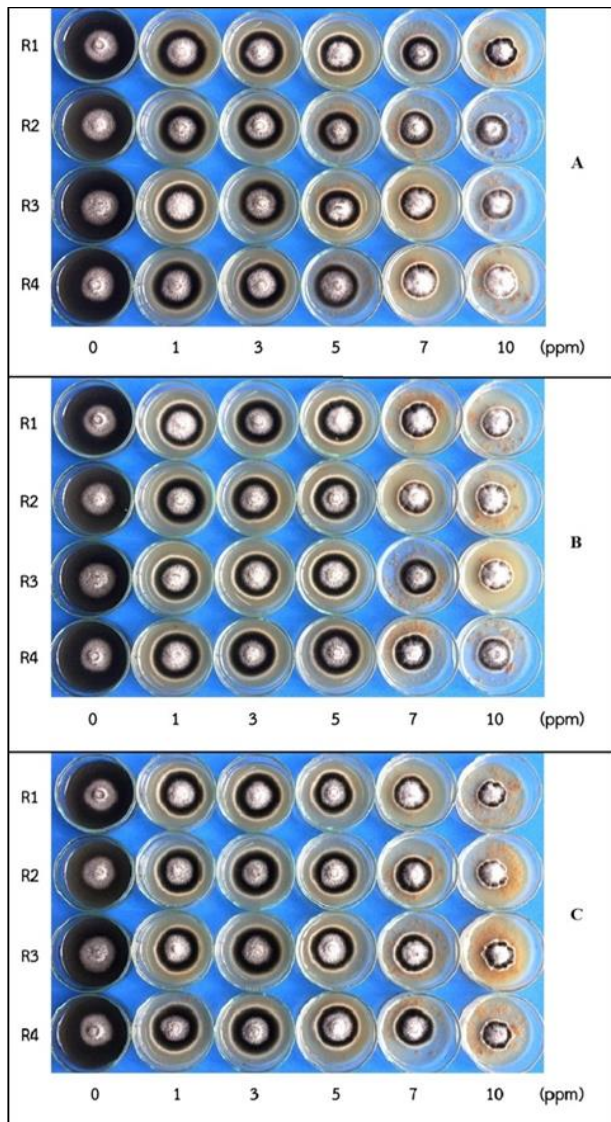| Crude extracts	Conc. (ppm)	spore number (10^5)	inhibition (%)	ED$_{50}$ (ppm)
Hexane	0	3.01^a	0.00^e	-
	10	1.68^{cde}	43.53^{cd}	
	50	1.78^{cd}	38.82^{cd}	
	100	1.94^c	34.86^d	
	500	1.86^{cd}	36.84^d	
	1000	1.53^{cde}	48.76^{cd}	
Ethyl acetate	0	3.01^a	0.00^e	544.44
	10	2.50^b	15.33^e	
	50	2.66^{ab}	9.87^e	
	100	1.78^{cd}	40.09^{cd}	
	500	1.44^{def}	50.86^{bcd}	
	1000	1.28^{ef}	55.91^{bc}	
Methanol	0	3.01^a	0.00^e	1.71
	10	0.99^f	66.66^b	
	50	0.24^g	91.55^a	
	100	0.14^g	95.12^a	
	500	0.10^g	96.37^a	
	1000	0.09^g	96.94^a	
	C.V. (%)	18.82	25.37	

$^{1/}$ *Means of four replications, means followed by the same letters are not significantly differed by DMRT at P =0.005*

Table 2. Growth inhibition of *Drechslera oryzae* treated with nanoparticles constructed from *Neosartorya hiratsukae* strain MST

Nano-particles	Conc. (ppm)	Colony dia. (cm.)	Inhibition (%)	ED$_{50}$ (ppm)
Nano-NHH	0	5.00^{al}	0.00^i	8.70
	1	3.57^b	28.50^g	
	3	3.42^c	31.50^g	
	5	2.97^d	40.50^f	
	7	2.52^f	49.50^d	
	10	2.27^g	54.50^c	
Nano-NHE	0	5.00^{al}	0.00^i	5.28
	1	2.97^d	40.50^f	
	3	2.92^{de}	41.50^{ef}	
	5	2.92^{de}	41.50^{ef}	
	7	2.22^g	55.50^c	
	10	1.99^h	60.00^b	
Nano-NHM	0	5.00^a	0.00^i	4.73
	1	3.00^d	40.00^f	
	3	2.97^d	40.50^f	
	5	2.80^e	44.00^e	
	7	2.29^g	54.00^c	
	10	1.79^i	64.00^a	
	C.V. (%)	3.09	5.02	

$^{1/}$ *Means of four replications, means followed by the same letters are not significantly differed by DMRT at P =0.005*

Table 3. Spore inhibition of *Drechslera oryzae* treated with nanoparticles constructed from *Neosartorya hiratsukae* strain MS

Nano-particles	Conc. (ppm)	Spore number (10^5)	Inhibition (%)	ED_{50} (ppm)
Nano-NHH	0	4.23[al]	0.00[h]	4.60
	1	3.56[b]	15.71[g]	
	3	3.06[cd]	27.26[ef]	
	5	2.80[de]	33.79[de]	
	7	1.31[g]	68.89[b]	
	10	0.47[h]	88.74[a]	
Nano-NHE	0	4.23[a]	0.00[h]	4.11
	1	3.34[bc]	20.75[fg]	
	3	3.35[bc]	20.27[fg]	
	5	2.32[f]	44.84[c]	
	7	1.24[g]	70.66[b]	
	10	0.28[h]	93.32[a]	
Nano-NHM	0	4.23[a]	0.00[h]	4.63
	1	3.53[b]	15.94[g]	
	3	3.52[b]	16.51[g]	
	5	2.56[ef]	39.53[cd]	
	7	1.44[g]	65.69[b]	
	10	0.22[h]	94.87[a]	
	C.V. (%)	9.06	14.63	

[1/] *Means of four replications, means followed by the same letters are not significantly differed by DMRT at P =0.005*

Table 4. The efficacy of nano-NHM from *Neosartorya hiratsukae* to control brown leaf spot of rice in 28 days

Disease index				
Treatments	days 7	days 14	days 28	(%) Disease reduction
T1) Inoculate control)	4.50[al]	3.50[a]	6.00[a]	-
T2 (nano-NHM)	4.75[a]	4.50[a]	4.00[b]	33.3
T3 (Carbendazim)	4.50[a]	4.50[a]	4.00[b]	33.3
CV%	12.06	39.19	11.17	-

[1/] *Means of four replications, means followed by the same letters are not significantly differed by DMRT at P =0.005*

Table 5. The effect of nano-NHM from *Neosartorya hiratsukae* for plant growth of rice var Korkor1 at 28 days

Treatments	tillers	plant height and roots	fresh weight of plant height and roots	dried weifght of plant	dried weight bof rots
T1) Inoculate control)	11.75[ab]	87.25[b]	27.07[b]	4.05[b]	0.86[ab]
T2 (Nano-NHM)	16.50[a]	102.00[a]	37.15[a]	5.39[a]	1.53[a]
T3 (Carbendazim)	10.50[b]	78.25[b]	22.48[c]	2.85[c]	0.73[b]
CV%	22.01	6.96	7.67	13.16	37.76

[1/] *Means of four replications, means followed by the same letters are not significantly differed by DMRT at P =0.005*

4. CONCLUSION

Neosartorya hiratsukae proved to be suppressed *Drechslera oryzae* (brown spot of rice). It is effectively inhibited the inocula of *D. oryzae*. The crude metabolite of *N. hiratsukae* is extracted by methanol showed highly antifungal activity to control inhibit the growth and sporulation of *D. oryzae* which the ED_{50} were 1.7 and 92 ppm., respectively. The biodegradable natural product of nanoparticles, SH-M expressed the highest inhibition of pathogen's sporulation at the ED_{50} of 4 ppm. The nanoparticles constructed from *N. hiratsukae* proved to be effective controlled rice brown spot in pot trials and gave a better plant stands than the control. It has further investigated to develop as a new biological product for plant disease control.

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