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Geo Referenced Plant Pathology Digital Herbarium – An Alternate Approach

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Abstract

Rice cultivation in Madurai, Tamil Nadu, India were under serious threat to two major disease namely rice blast caused by *Magnaporthegrisea* and brown spot of rice caused by *Helminthosporiumoryzae*. Documentation of these two disease carried out in 30 locations of Madurai along with Geo positioning System for precise location of disease occurrence. Three different disease spreading patterns observed. Resistant varieties ofKarnataka Ponni, Super Ponni, ADT 36, ASD 16 , and ASD 19 both blast and brown spot of rice were found to be affected severely. *In vivo* attempt was made to control these disease with *Pseudomonos* species. The alarming study definitely gains a greater importance to rice resistant breeders. This pilot study would be extended to field study in future.

Key Words: *Magnaporthegrisea, Helminthosporiumoryzae* and *Pseudomonos*

1. Introduction

All over the world the plant pathogens are serious threat to plants and crops at all time. For a better disease management through study is needed about the pathogens origin, development and disease spread pattern. To achieve this a survey study on every crop at different seasons is to be carried out. Our present study about the major disease of rice would definitely have a great impact on rice disease management. Major food crops survey study is in priority.

Oryzasativa L. (Rice) a predominant crop occupies 90% in Asia 60% in the

world population food (Kole, 2006). This valuable crop is always under serious threat to many disease among those the rice blast disease caused by *Magnaporthegrisea* gains extensive pecuniary importance (Kapoor Pooja & Abhishek Katoch, 2014). The symptoms rice blast appears pin head spots with grey lesions. Older lesions on the leaves are elliptical with grey centres with brown border.

From the year of 1994 the rice blast destroys feed of 60 million peoples world wide (Zeigler Leong,&Teng 1994). According to Ou 1971 the rice blast disease has its own disease spreading pattern stated in China in 1637, Japan in 1704, Italy 1828, USA 1876 and in India in 1913.

In India the spread of rice blast in India recorded during 1913 and these disease occurred as epidemic in Tanjore of Tamil Nadu (Padmanabhan 1965). This epidemic proposition recorded in Himachal Prdesh, Andhra Pradesh, Haryana and Tamil Nadu (Nagarajan 1988).

Farmers of Madurai region are enduring losses in yield due to Rice Blast disease. Complaints were received from farmers group regarding the loss of their crop because of these disease.

As important as rice blast another major prolonging disease of rice is Brown spot of rice caused by *Helminthosporiumoryzae*. With the symptoms of brown spot of rice is rectangular or irregular brown spots appear on leaf blades and leaf sheaths later the spots develops in to ellipsoidal or oval or circular spots. The advance lesions appear with reddish brown margin with light brown centre.

This wide spread disease reported almost all rice cultivating regions of India reported from West Bengal (Bengal famine 1942) to Tamil Nadu. Its yield loss, grain loss, epiphytotic, annual loss and leaf spotting phase devastation has been reported by many workers like Ghosh et al., 1960;Padmanabhan (1973); Bedi and Gill (1960); Kawada et al., (1954).

Apart from Pathogenesis the rice blast fungi *Magnaporthegrisea* produces tenuazonic acid a mycotoxin (Steyn &Rabie 1976; Umetsu et al., 1972). Which plays key role in protein bio synthesis inhibition and by inhibiting the release of new protein (Shigeru& Gordon 1963). Recently the biosynthetic gene was identified by Choong-Soo Yun et al., in 2015

Similarly the *Helminthosporiumoryzae* is also producing mycotoxins called ophiobolin A, chchliobolin A This toxin reduces the germination of seeds and produces irregularities in seeds growth. (Canonica et al., 1966; Nakamura et al., 1958;Orsenigo et al., 1956, 1957).

Therefore the present survey study emphasised in major rice cultivating regions of Madurai to study the disease occurrence and severity of Rice blast and Brown spot disease in different rice varieties, agronomic practices, agro-ecosystems and cropping systems. This survey study would definitely assist sustainable disease management. Our present Objectives are: Pathological

survey to all major rice growing regions of Madurai, Passport data collection of variety name, Place, Water source, Farmers data etc.,GPS based survey , Isolation of rice blast and brown spot pathogen, Isolation of *Pseudomonos* from rhizosphere soil, Antagonistic activity of *Pseudomonos* against *Magnaporthegrisea* and *Helminthosporiumoryzae*

2. Materials and Methods

2.1. Pathological survey to all major rice growing regions of Madurai

Pathological survey was made from all major rice growing regions of Madurai and Sivagangai. Covering all four directions at different stage of crop. Sakkimangalam, Silaiman, Keeladi, Viradhanoor, Sathyanagar, Pottapalayam, Chinthamani, Viraganoor - Paniyoor Road, Thiruparankuntram, Thirumangalam, Nagamalaiputhukottai, Samayanaloor, Ayyankottai, Vaddipatti, Alanganaloor, Vavidamaruthur, Karuvanoor, Velichanatham - Karuvanoor Road, Natham Road, Kodikulam, Chittakur, Puthupatti, Narasingam, Thirumohur, Thirukanai, Velaripatti, Amoor, Perakkur, Thiruvathavur, and Melur.

2.2. Passport data collection of variety name, Place, Water source, Farmers data etc.,

Data sheets were used to record the details such as cultivar, place of collection, date of collection, water source, famers name, Harvest time, Name of the pathogen, soil type, fertilizers used and chemicals sprayed.

2.3. GPS based survey

Every survey area is measured with Geo Position System based survey measuring Latitude and Longitude.

2.4. Isolation of rice blast and brown spot pathogen

The collected samples were surface sterilized with 0.1% sodium hypochlorite. And transferred to Petri dish moist chamber for sporulation at 24hrs at 22° C. After sporulation the pyri-formed spores were confirmed in microscope and transferred to PDA Medium[Potato Dextrose Agar Medium]. PDA Medium Ingredients. Potato - 200g; Dextrose -20g; Agar-20g; Distilled water -1L

2.5. Isolation of Pseudomonos from rhizosphere soil

Rhizosphere soil samples were collected from rice field Keeladi and Sakkimangalam. Dilution plate techniques were followed, with nutrient agar medium (NA). The soil suspension were taken and diluted to 10^3-10^5 x in distilled water. The diluted soil suspensions poured on petri dishes containing Kings B agar medium and incubated at room temperature for 24^0c / 48h. At the end of the incubation, different types of bacterial colonies that appeared on the medium will be selected and re streaked for pure culture. In pure culture techniques four way streak method is followed. Composition of Kings B Agar Medium: protease peptone - 20g; Di-potassium hydrogen phosphate -1.5g; Magnesium sulphate - 1.5g; Agar -20g; Glycerol -15g ;pH -7.2.

2.6. ***Antagonistic activity of Pseudomonos against Magnaporthegrisea and Helminthosporiumoryzae***

Using petri dishes for dual plate assay to examined the antagonistic activity of isolated bacterial strain against isolated fungus. The dual plates were incubated in room temperature for 4-7 days and examined the bacterial strains for their antagonistic activity against fungus (*Puriculariaoryzae* and*Hilminthosporiumoryzae*).The strains which shows the zone of inhibition in 1cm or more than that ,it taken for further studies.

3. Results

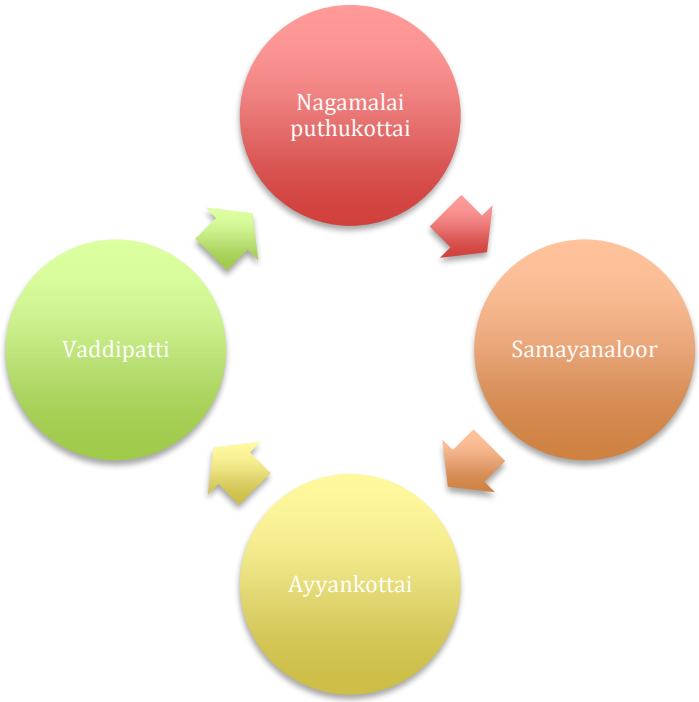
3.1. Pathological survey to all major rice growing regions of Madurai

The survey results recorded rice blast disease occurrence in Sakkimangalam, Pottapalayam, Chinthamani, Thiruparankuntram, Nagamalaiputhukottai, Samayanaloor, Ayyankottai, Vaddipatti, Velichanatham - Karuvanoor Road, Narasingam, Thirumohur, Thiruvathavur, and Melur. The pattern of disease spread appears as a chain link starts from Sakkimangalam, Pottapalayam, Chinthamani, Thiruparankuntram. Distance cycle map indicates Sakkimangalam, to Pottapalayam– 9KM; Pottapalayam to Chinthamani- 7.1KM;Chinthamani to Thiruparankuntram-9.7Km. An average of 8.3KM distance the pathogen revolves around this area. (Figure 1)

Figure 1. Disease spread cycle between Sakkimangalam, Pottapalayam, Chinthamani, 9. Thiruparankuntram regions

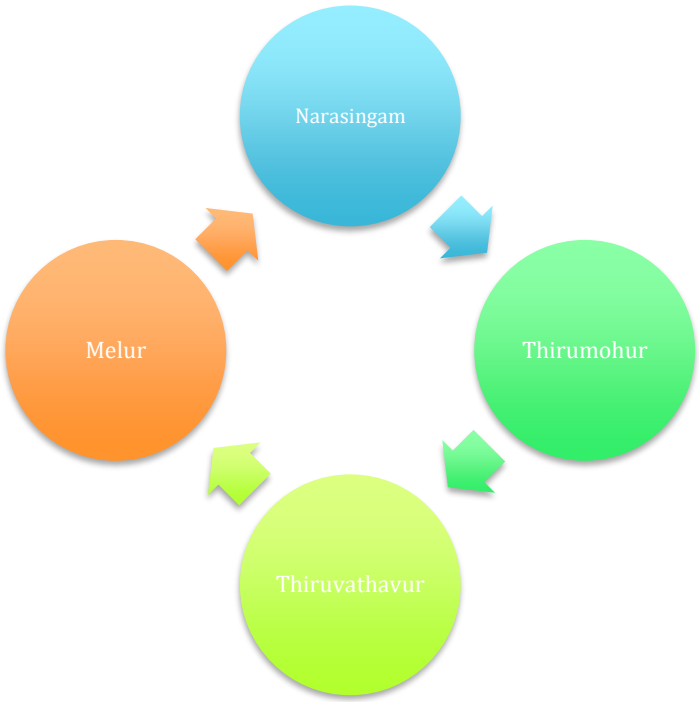
Another pattern observed from Nagamalaiputhukottai, 11.3 km Samayanaloor, 8.3KM Ayyankottai, 9KM Vaddipatti. An average of 9.5KM distance the pathogen revolves around this area. (Figure 2).

Figure 2. Disease spread cycle between Nagamalaiputhukottai, Samayanaloor, Ayyankottai and Vaddipatti regions



Followed from Narasingam to Thirumohur - 5.4KM; Thirumohur to Thiruvathavur-12.9 KM, and Thiruvathavur to Melur-9.3KM. An average of 9.2KM distance the pathogen revolves around this area. (Figure 3).

Figure 3. Disease spread cycle between, Narasingam, Samayanaloor, Thirumohur, Thiruvathavur and Melur regions.



These pattern is due to inter connected water channel runs between the connected regions for example Sakkimangalam (Sakkimangalamkanmai), Pottapalayam (Rain water), Chinthamani (Chinthamanikanmai) and Thiruparankuntram (Thenkarai pond) all these inter connected water channels acted as a carrier for spreading the disease. Interestingly all the above said places has same type of soil conditions namely alluvial soil. This soil factor may also facilitated the same disease spread. (Figure 4)

We also observed that rice blast symptoms first recoded in Chinthamani during Last week December. When the crop is at 20 days old followed by in Pottapalayam during 1st week of Jan. 26 d old crop, Sakkimangalam during 1st week of Feb. 32 d old crop and in Thiruparankuntram during 2st week of Feb. 21 d old crop. These disease precedence indicates the movement of *M.grisea* from one place to another in periodical manner respective to its conducible climate (Dec to Feb) irrespective of variety of rice. (Table. 1)

Rapidity disease spread observed in Nagamalaiputhukottai, Samayanaloor, Ayyankottai and Vaddipatti regions. During the month of Jan and Feb all the above said regions were affected by rice blast pathogen when the crop stand at same stage in all regions irrespective of variety. Almost similar pattern observed in Narasingam, Thirumohur, Thiruvathavur, and Melur regions.

When its come to variety choice Akshya, Swarna, LLR and Culsar were severely affected showing disease severity up to 7 IRRI scale. Urutu, Nlr, ADT 16, IR 20 recorded moderate infection scale 5 where as Karnataka Ponni, Super Ponni showed resistant score up to 2 to 3 IRRI scale. Besides the resistant varieties of ADT 36, ASD 16 , and ASD 19 were also found to be infected by the *Magnaporthegrisea* pathogen this urgent issue must be attended as earliest. (Figure 4.1& Figure 4.2)

Brown spot of rice occurred in Silaiman, Keeladi, Viradhanoor, Sathyanagar, Viraganoor - Paniyoor Road, Thirumangalam, Alanganaloor, Vavidamaruthur, Natham Road, Kodikulam, Chittakur, Puthupatti, Thirukanai, Velaripatti, Amoor and Perakkur,

Since brown spot of rice is seed born disease its emerge from the seed (Ou 1985) and show symptoms in plants during the tillering stage of the plant.

The most affected places are Sathyanagar, Thirumangalam, Chittakur, Putthupatti Moderate infections observed in Silaimaan, Viraganoor-Panaiyur Road. The most affected varieties are Telex, ADT 16. Moderate infections observed in Culsar, Jeyganesh and urutu varieties

Table 1. Passport data of pathological survey of Rice Blast and Brown Spot of rice

Sl. No.	Place	Variety	Month	Time for Harvest	Disease Identified	Causal Organism	Water source	Soil	Chemicals Used	Nutrient and Fertilizer	Data Source
1	Sakkimangalam	Karnataka Ponni, Akshaya	Feb	5 weeks	Blast	*Magnaporthegrisea*	Sakkimangalamkanmai	Karambaivandal	Cypermethrin	Urea, Azotobacter	Muthupandi, Farmer Native
2	Silaimaan	Culsar, IR 20	Jan	4 weeks	Brown spot	*Heliminthosporiumoryzae*	Vaigai river	Savadu, Karambai	Karate Carbendazium	Urea, Azotobacter	Dharmarasu, Farmer Native
3	Keeladi	Adt 36,Nlr	Jan	4 weeks	Brown spot	*Heliminthosporiumoryzae*	Bene, Rain water	Savadu, Karambai	Propanil	Urea, Azotobacter	Dharmarasu, Farmer Native
4	Viradhanoor	Nlr	Jan	4 weeks	Brown spot	*Heliminthosporiumoryzae*	Bene, Rain water	Vandal	Bentazon, Propanil	Urea, Azotobacter	Murugan, Farmer Native
5	Sathyanagar	Telex	Feb	2 weeks	Brown spot	*Heliminthosporiumoryzae*	Bene, Rain water	Karambai	Metotrino-Strobin	Urea, Azotobacter	Murugan, Farmer Native
6	Pottapalayam	Tkn13	Jan	6 weeks	Blast	*Magnaporthegrisea*	Bene, Rain water	Karambai	Karate	Urea, Azotobacter	Karungan, Farmer
7	Chinthamani	Karnataka Ponni	Dec	7 weeks	Blast	*Magnaporthegrisea*	Chinthamani pond	Karambai	Cypermethrin, Metotrino-	Urea, Azotobacter	Mohan, Farmer

									strobin		
8	Viraganoor-Panaiyur Road	Urutu	Jan	6 weeks	Brown spot	*Heliminthosporiumoryzae*	Iravathanalur pond	Savadu, Karambai	Malathion	Urea, Azotobacter	Mohan, Farmer
9	Tiruparankundram	Ponni,Asd 16	Feb	4 weeks	Blast	*Magnaporthegrisea*	Thenkarai pond	Karambaivandal	Cypermethrin	Urea, Azotobacter	Meenakshi, Farmer Native
10	Thirumangalam	45,Adt 16	Feb	2 weeks	Brown spot	*Heliminthosporiumoryzae*	Gundaru river	Karambai	Karate, Carbendazium	Urea, Azotobacter	Kannan, Farmer
Sl. No.	**Place**	**Variety**	**Month**	**Time for Harvest**	**Disease Identified**	**Causal Organism**	**Water source**	**Soil**	**Chemicals Used**	**Nutrient and Fertilizer**	**Data Source**
11	Nagamalaiputhukottai	Ir 18,Ir 20	Jan	3 weeks	Blast	*Magnaporthegrisea*	Bene,Rain Water	Red Soil	Cypermethrin, Karate	Urea, Azotobacter	Ramalaksmi,Farmer Naïve
12	Samayanaloor	Ponni, Akshya	Jan	3 weeks	Blast	*Magnaporthegrisea*	Thodaneri Pond	Karambai	Cypermethrin, Metotrino-Strobin	Urea, Azotobacter	Sivakumar, Farmer Native
13	Ayyankottai	Swarna, Karnataka Ponni	Feb	3 weeks	Blast	*Magnaporthegrisea*	Ayyankottai pond	Karambai	Malathion, Cypermethrin	Urea, Azotobacter	Pandi, Farmer
14	Vadipatti	Urutu,51, Nlr	Feb	3 weeks	Blast	*Magnaporthegrisea*	Puliyan&ThathapaNayaka	Karambai	Cypermethrin,	Urea, Azotobact	Pandi, Farmer

							mpatty pond		Metotrinostrobin	er	
15	Alanganaloor	45, Asd 19	Dec	4 weeks	Brown spot	*Heliminthosporiumoryzae*	Bene,Rain Water	Savadu, Karambai	Malathion	Urea, Azotobacter	Janarthanan, Farmer Native
16	Vavidamaruthur	Mdu4, Asd 16	Oct	3 weeks	Brown spot	*Heliminthosporiumoryzae*	Vavidamaruthur Irrigation Tank	Savadu, Karambai	Carbaryl, Cypermethrin	Urea, Azotobacter	Elongo, Farmer
17	Karuvanoor	Super Ponni, Adt 38	Feb	4 weeks	Brown spot	*Heliminthosporiumoryzae*	Karuvanoor Irrigation Tank	Savadu, Karambai	Karate, Carbendazium	Urea, Azotobacter	Elongo, Farmer
18	Velichanatham-Karuvanoor Road	Karnataka Ponni, Ir 8	Feb	4 weeks	Blast	*Magnaporthegrisea*	Bene,Rain Water	Vandal, Karambai	Cypermethrin, Metotrinostrobin	Urea, Azotobacter	Elongo, Farmer
19	Natham Road	Ir 8	Oct	4 weeks	Brown spot	*Heliminthosporiumoryzae*	Bene,Rain Water	Vandal ,Karambai	Carbendazium	Urea, Azotobacter	Velmurugan, Farmer Native
20	Kodikulam	KaranatakaPonni	Feb	5 weeks	Brown spot	*Heliminthosporiumoryzae*	Kodikulum Irrigation Tank	Red Soil	Karate, Malathion	Urea, Azotobacter	Velmurugan,Farmer Native

Sl. No.	Place	Variety	Month	Time for Harvest	Disease Identified	Causal Organism	Water source	Soil	Chemicals Used	Nutrient and Fertilizer	Data Source
21	Chittakur	Jeyganesh	Feb	2 weeks	Brown spot	*Heliminthosporiumoryzae*	Bene,Rain Water	Karambai	Carbendazium, Carbaryl	Urea, Azotobacter	Ramalaksmi,Farmer Naïve
22	Putthupatti	Urutu	Jan	4 weeks	Brown spot	*Heliminthosporiumoryzae*	Bene,Rain Water	Vandal, Karambai	Carbendazium, Carbaryl	Urea, Azotobacter	Sivakumar, Farmer Native
23	Narasingam	superponi, Adt 16	Feb	3 weeks	Blast	*Magnaporthegrisea*	NarasingamIrragation Tank	Karambai	Cypermethrin, Metotrinostrobin	Urea, Azotobacter	Pandi, Farmer
24	Thirumohur	Adt 16	Feb	4 weeks	Blast	*Magnaporthegrisea*	Thirumohur Lake	Red Soil	Malathion, Metotrinostrobin	Urea, Azotobacter	Pandi, Farmer
25	Thirukanai	Poni, swarna	Feb	3 weeks	Brown spot	*Heliminthosporiumoryzae*	ThirukanaiIrrugation Tank	Vandal	Carbaryl, Cypermethrin	Urea, Azotobacter	Janarthanan,Farmer Native
26	Velaripatti	Asd 19, Super poni	Feb	5 weeks	Leaf streak	*Xanthomonosoryzae*	Velaripatti Irrigation Tank	Savadu, Karambai	Carbendazium, Carbaryl	Urea, Azotobacter	Elongo, Farmer
27	Amoor	karnataka poni, Akshaya	Feb	3 weeks	Brown spot	*Heliminthosporiumoryzae*	Amoor Pond & Bene	Karambai	Carbaryl, Malathion	Urea, Azotobacter	Elongo, Farmer

28	Perakkur	Ir 16	Feb	3 weeks	Brown spot	*Heliminthosporiumoryzae*	Perakkur Pond& Bene	Karambai	Karate, Cypermethrin	Urea, Azotobacter	Elongo, Farmer
29	Thiruvathvur	Nlr, Asd 16	Feb	4 weeks	Blast	*Magnaporthegrisea*	ThiruvathavurLake,Upparu River	Karambai	Metotrinostrobinkarate	Urea, Azotobacter	Velmurugan,Farmer Native
30	Melur	Akshya ,Llr	Jan	7 weeks	Blast	*Magnaporthegrisea*	Surrakundu Lake	Vandal, Karambai	Malathion	Urea, Azotobacter	Velmurugan,Farmer

3.2. *Passport data collection of variety name, Place, Water source, Farmers data*

Survey data needs a through study about the all available information. Our study report passport data collection of rice pathological survey projected the following results. The place of collection sites clearly illustrated that rain fed areas of rice cultivation is shrinking because of unseasonal rains. Among 30 sites only 10 sites depends on rain fed cultivation. (Table. 1)

When it comes to variety selection farmers always adopt high yielding short varieties like Karnataka Ponni, super ponni and Culsar. The age old ethnic varieties of Madurai like IR 8 and IR 20 were very hard to find in the field like Silaiman, Vadipatti, Natham and Puthupatti villages.

The parameters of month of cultivation is majorly depends only on the water availability time. Previously in the age old record Madurai reported to cultivate 3 times in a year but prolonged rain failure it decreased in to once in a year that to during December to February.

The major water source of Madurai is vaigai river prevailing water scarcity in the river decreased rice cultivation throughout its river bed except in few places like Silaiman and Sakkimangalam. All the rest of cultivation depends majorly on water pond or small river stream.

Disease note resulted 14 places with rice blast fungi and rest 16 places with brown spot of rice. The Karnataka Ponni said to be tolerant to rice blast disease recorded symptoms from areas like Sakkimangalam, Chinthamani, Ayyankottai and Velichanatham - Karuvanoor Road.

The prevailing strategies of using chemicals against both disease yielded only 50% results. As Cypermethrin, Metotrinostrobin, Malathion, Carbaryl and Cypermethrin are broad spectrum fungicides over many varieties our results projected the efficiency of these fungicides is not 100%. The remaining 50% of disease inoculum is sufficient to cause disease in random proportional. Though our farmers used Azotobacter as nutrient supplement these activity was nullified due to chemicals predisposition in soil.

3.3. GPS based Survey

The Geo Positioning System assisted survey introduced in the survey in order to have a clear location site where the pathogen existed. By measuring the latitude and longitude of North and East along with altitude in meters the clear location of pathogen can be tracked. The disease altitude of rice blast ranges from 119 to 177m and brown spot of rice ranges from 119 to 186m. (Table. 2)

Table 2. Geo Positioning System based Pathological disease survey

S.NO	Place	GPS CODE	Altitude	Pathogen
1	Sakkimangalam	$9^0$53'12"N $78^0$11'25"E	119m	*M.Grisea*
2	Silaimaan	$9^0$52'13"N $78^0$11'11"E	119m	*H.oryzae*
3	Keeladi	$9^0$52'10"N $78^0$11'12"E	118m	*H.oryzae*
4	Viradhanoor	$9^0$48'54"N $78^0$10'35"E	114m	*H.oryzae*
5	Sathyanagar	$9^0$49'31"N $78^0$10'34"E	119m	*H.oryzae*
6	Pottapalayam	$9^0$50'45"N $78^0$10'21"E	119m	*M.Grisea*
7	Chinthamani	$9^0$53'14"N $78^0$08'11"E	130m	*M.Grisea*
8	Viraganoor-Panaiyur Road	$9^0$53'59"N $78^0$01'36"E	124m	*H.oryzae*
9	Thiruparankuntram	$9^0$54'32"N $78^0$03'42"E	141m	*M.Grisea*
10	Thirumangalam	$9^0$49'23"N $77^0$58'27"E	123m	*H.oryzae*
11	Nagamaliputhukottai	$9^0$36'49"N $77^0$03'36"E	144m	*M.Grisea*
12	Samayanaloor	$9^0$59'21"N $78^0$01'49"E	154m	*M.Grisea*
13	Ayyankottai	$10^0$02'27"N $78^9$00'13"E	176m	*M.Grisea*
14	Vadipatti	$10^0$04'36"N $77^0$51'38"E	186m	*M.Grisea*
15	Alanganaloor	$10^0$02'35"N $78^0$05'32"E	177m	*H.oryzae*
16	Vavidamaruthur	$10^0$02'19"N $78^0$07'43"E	170m	*H.oryzae*
17	Karuvanoor	$10^0$01'02"N $78^0$08'38"E	145m	*H.oryzae*
18	Velichanatham-Karuvanoor Road	$10^0$00'51"N $78^0$09'04"E	153m	*M.Grisea*
19	Natham Road	$10^0$00'12"N $78^0$09'10"E	145m	*H.oryzae*
20	Kodikulam	$10^0$00'02"N $78^0$09'32"E	146m	*H.oryzae*
21	Chittakur	$9^0$56'46"N $78^0$15'39"E	117m	*H.oryzae*
22	Putthupatti	$9^0$59'19"N $78^0$12'36"E	149m	*H.oryzae*
23	Narasingam	$9^0$58'23"N $78^0$10'27"E	141m	*M.Grisea*
24	Thirumohur	$9^0$57'05"N $78^0$12'33"E	131m	*M.Grisea*
25	Thirukanai	$9^0$57'09"N$78^0$16'25"E	128m	*H.oryzae*
26	Velaripatti	$9^0$56'31"N $78^0$16'18"E	117m	*H.oryzae*
27	Amoor	$9^0$56'28"N $78^0$15'55"E	124m	*H.oryzae*
28	Perakkur	$9^0$57'34"N $78^0$15'09"E	130m	*H.oryzae*
29	Thiruvathvur	$9^0$58'18"N $78^0$19'28"E	113m	*M.Grisea*
30	Melur	$10^0$02'33"N $78^0$19'28"E	147m	*M.Grisea*

3.4. Isolation of rice blast and brown spot pathogen

Pyriformed spores of *Magnaporthegrisea* were confirmed by observing under microscope. (Figure 5) And Dark brown two to four septate swollen cell ay base shape of spore of *Helminthosporiumoryzae* is also confirmed by micro-scopical studies. (Figure 6)

3. 5.Isolation of Pseudomonos from rhizosphere soil

Fluorescent *Pseudomonos* were observed in Kings B medium after 24 hrs of incubation. The microscopic observation of *Pseudomonos* were with rod shaped bacteria and the wall layer of the cell were stained in red colour indicating the Grams negative nature. (Figure 7)

3.6.Antagonistic activity of Pseudomonos against Magnaporthegrisea and Helminthosporiumoryzae

The fungal mycelial growth of *Magnaporthegrisea*and *Helminthosporiumoryzae* were supressed by rhizosphere bacteria Pseudomons. (Figure 8a,b,9a,b)

4. Discussion

Rice disease management system started from its crop cultivation but still there were many rice disease problems exist. All the control strategies of Physical, Chemical, Biological and Genetic control were not persistent because of the continuous emergence of virulence of the pathogen in every five years. Disease monitoring studies, disease containment strategies, disease reporting at early time are the age old practices but need of the hour study. This un avoidable study always stands in precedence.

Apart from pathological problem the capability of these two fungi producing mycotoxins are a serious threat to food and agriculture system. As these mycotoxins contaminated the food chain and proven to cause cancer in human and animals (Charmley, et al., 1995; Cheeke, et al., 1995; Fink-Gremmels, et al, 1996; Kuiper-Goodman, et al., 1998; Massey, et al., 1995).

Though there were many reports on rice blast and brown spot of rice and its management through out the world our study reports states that still the blast and brown spot of rice is not all eradicated form the formers field. During the survey study we observed the disease spread pattern. Pattern of rice blast disease spread as observed by Suriya Rao and ParsuramNayakin 2007. We recorded that there were only around 9 KM of distance the rice blast pathogen surrounds the area and reclaiming its infection cycle during the month of December to February (28° C)for prolonging years. This phenomenon of rapidity indicates the climate utilization of pathogen with short span of time. (AndréVelásquez, et al.,

2019; Yigalelad and IlariaPertot, 2014) was coincided with our repot of study.

Another alarming issue observed in our study is the so called rice blast resistant varieties like Karnataka Poni, Super Ponni, ADT 36, ASD 16 , and ASD 19 were found to be infected by *Magnaporthegrisea*.

Resistance or tolerance level breakdown urgent study is needed in this issue because of the resistance breakdown is rapidly occurring in Karnataka ponni variety. This pioneer study report may open up many research thoughts like pathogenicity analysis and virulence gene analysis. The prolonged usage of chemicals like Cypermethrin, Metotrinostrobin, Malathion, Carbaryl and Cypermethrin left its chemical debris in soil by the way killing the beneficial soil microflora.

The seed born infection of brown spot can be eliminated only by selecting disease free seeds. Brown spot is also air born or water carrier or by contact with infected ones. These seed born infection of brown spot of rice is considered to be a harmless issue. But out work stands a examples that this infection spreads from seed to seed by the its prolongs its life cycle and gaining its virulence over many improved varieties.

Due to unseasonal rains many rice cultivating areas were transformed into millet, sorghum cultivation or even farmers left the land for construction. In addition the major pathogens of rice also causes high crop damage.

The major cause of cultivating new high yielding varieties like Karnataka Ponni, Super ponni and Culsar in-between the field of high susceptible varieties like swarna and Akshaya will definitely increase the chance of pathogen gaining virulence. Besides the modern varieties will eliminate the native varieties like Urutu and IravaiPandi is almost eradicated from the field. Water scarcity and decreasing of rice cultivation in Madurai.

We observed a critical study in Urakundu site near sakkimangalam, Madurai where almost 100 acres of rice cultivation in one place purely depends Pond irrigation. In this large field farmers were cultivating almost 7 to 10 different rice varieties it includes susceptible swarna and Akshaya along with tolerant variety like Karnataka and super ponni. This community pooling of rice varieties increases the virulence level of pathogen.

When karnatakaPonni was released from Karnataka to Tamil Nadu it was free from any seed born infections. But in our study we observed Karnataka Ponni of Kodikulam and Amoor was infected by brown spot of rice. This would be the reasons of the particular famer would have stored

healthy seeds along with infected ones this cross contamination of seeds resulted in emergence of brown spot disease in Karnataka Ponni. This peculiarity disease spread will continue as community spread like corona and makes Karnataka Ponni as infected ones in future. This precursor study gains its key role in epidemiology.

Another part of our study inclines with the usage of GPS data determines exact location and these information system is widely used in agriculture data. For plant pathology purpose the disease occurrence and spread of *Aspergillus flavus* in tomato, Tomato virus management, Cotton leaf curl disease and *Phytophthorainfestans* was studied by Merritt et al., in 1999. Followed by Thomas et al., in 2002 utilized GPS technology for outbreak study of 12 diseases and insect and pest problems in various crops. The GPS technology is first used in rice blast disease from Kenya by Joseph Kihoro et al., in 2013. Up to our knowledge form India GPS based rice blast and brown spot of rice survey was so not yet done. Our study work may stands as pioneer approach on blast and brown spot of rice disease.

For better disease management biological control is a proven effective approach in that the*Pseudomons* produces 2,4, Diactylfluroglucinol key component for inhibition of fungal growth. The growth suppression of *M.grisea* was already reported by Karthikeyanand Gananmanicakm, 2008; and suppression of *H.oryzae* by *Pseudomons* also reported by Balgude, et al., 2017. Our results also supports the previous reports on biological control of rice diseases. In future this studies may be extended to field studies.

The regions like Madurai, Tamil Nadu, India mainly dependents on rice cultivation and the rice straw stands as a major fodder to cow and bulls. We recorded the rice varieties cultivated in Alaganallur place famous for bulls race in Tamil Nadu, India, were more affected by these two pathogens. If these two pathogens were not reported on the right time and if there any failure in controlling these two diseases these mycotoxins will definitely harm the cattle and bulls by causing Oesophagus Cancer and may lead to death of ethnic bulls of Madurai.

Our present study indicates the effect of and *H.oryzae* are still a major problem in Madurai breaking resistant genes and gaining its virulence and increasing mycotoxin problems.

The major crop rice prevails in this world since 5000 years ago. The challenges on rice cultivation is increasing from biotic and abiotic factors. Major pathogens of both fungi and bacteria also affecting the rice cultivation. As the disease control strategies evolves day by day the pathogen virulence also evolves. Disease detection at right time is the first

phenomenon of disease control strategy. Our work on survey of pathogens prevails in the rice field and addressing it on right time along with recent GPS technologies are greater importance to this present world. Biological control of rice disease are effective measures to save this age old crop.

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Reference:

1. Agrios G. N. 2005. Plant Pathology. Fifth Edition. Elsevier Academic Press, London, UK. 922 .Anonymous . Agriculture Situation in India.
2. Amutharaj P.,Sekar C., Esathnatheer, S. 2013. Development and Use of Different Formulations of *Pseudomonas flurescens* Siderophore for the Enhancement of Plant Growth and Induction of Systemic Resistance Against *Pyriculariaoryzae* in Lowland Rice Department of Microbiology, Faculty of Science, Annamalai University, India. Int J Pharm Bio Sci, 4(2):831-838.
3. André C., Velásquez Christian Danve M.,Castroverde., Sheng Yang. 2018.HePlant and pathogen warfare under changing climate conditions. CurrBiol, 28(10): 619–634.
4. Awla H. K., Rashid T. S., Hamid S., Wong, M. Y., 2017. Plant Growth Promoting Abilities and Biocontrol Efficacy of Streptomyces sp. UPMRS4 Against*Pyriculariaoryzae*, Biological Control. Theory and Applications in Pest Management, 01 Sep 2017, 112:55-63
5. Balgude Y. S., Gaikwad A. P., Sagar K., 2017. *Pseudomonas fluorescens*, a Potential Bioagent for Effective Management of Diseases in Organic Rice Production. Journal of Rice Research 2, 10:2
6. Bedi K. S., Gill H. S., 1960. Losses caused by Brown Leaf Spot Disease of rice in Punjab. 13:161-164.
7. Canonica L., Fiecchi A., Galli Kienle M., Scala A., 1966. The Constitution of Cochliobolin, Tetrahedron Lett.11:1211-1218.
8. Canonica L., Fiecchi A., Galli Kienle M., Scala, A., 1966. Isolation and Constitution of Cochliobolin B. Tetrahedron let. 13:1329-1333.
9. Charmley L., Trenholm H. L., Prelusky D. B., Rosenberg A. 1995. Economic Looses and Decontamination. Nat Toxins 3:199-203,221.
10. Cheeke P. R., 1995. Endogenous Toxins and Mycotoxins in Forage Grasses and Their Effects on Livestock. J AnimSci, 73:909-18.
11. Choong-Soo Yun., Takayuki Motoyama1., Hiroyuki Osada., 2015.Biosynthesis of the mycotoxin tenuazonic acid by a fungal NRPS–PKS hybrid enzyme Nature Communications. 6:8758:1-9.

12. Cu R. M., Mew T. W., Cassman K. G. Teng, P. S. 1996. Effect of Sheath Blight on Yield in Trophical, Intensive Rice Production System. Plant Disease, 80:1103-1108.
13. Duffy B. K. Defago G., 1999. Environmental Factors Modulating Antibiotic and Siderophore Biosynthesis by *Pseudomonas fluorescens* Biocontrol Strains. Apple Environ Microbiol, 65:2429-2438.
14. Felsenstein J., 1985. Confidence Limits on Phylogenies: An Approach Using the Bootstrap. Evolution. 39,783-791.
15. Ghosh R. L. M., Chatge M. B., Subramanyam V. 1960. Rice in India. Indian Council of Aar. Res. New Delhi.
16. Gnanamanickam S. S., Mew T.W., 1992. Biological Control of Blast Disease of Rice (*Oryzasativa* L.) with Antagonistic Bacteria and Its Mediation by a *Pseudomonas* Antibiotic. 58(3):380-385.
17. Gremmels J. F., Georgiou N. A., 1996. Risk Assessment of Mycotoxins For the Consumer. Residues of Veterinary Drugs and Mycotoxins in Animal Products, 159-74.
18. Kapoor Pooja., Abhishek Katoch., 2014.Recent Concepts in Fungal Taxonomy. 3 (2). Research & Reviews: Journal of Agriculture and Allied Sciences
19. Karthikeyan V.,Gnanamanickam S.S., 2008. Biological control of *Setaria*blast (*Magnaporthegrisea*) with bacterial strains. *Crop protection,* 27(2):263-267.
20. Kawada A., 1964. Insect and Disease of Rice in Japan. Tokva. Matt. Inot. Aar. Sci, P-33.
21. Kihoro J., Bosco N. J., Murage H., Makihara E. A. D., 2013. Investigating the Impact of Rice Blast Disease on the Livelihood of the Local Farmes in Greater Mwea Region of Kenya. Springerplus, 2(308):1-13.
22. Kole, C. 2006. Cereals and Millets . Springer,vol 1.
23. Kuiper Goodman T., 1998. Food safety: Mycotoxins and Phytotoxins in Perspective. Developments in Chemistry. Toxicology and Food Safety, 25-48.
24. Kulmitra A. K., Sahu N., Kumar V.B., Thejesha A.G., Ghosh A., Gulnaz Y.,2017. In vitro Evaluation of Bio Agents Against *Pyriculariaoryzae* (Cav.) Causing Rice Blast Disease. Agric. Sci. Digest,37(3):247-248.
25. Kumar S., A Lal A., Kumar N., Jaiswal S., Kumar H., Kumar A., Kumar M.,2017. Effect of Bio Control Agents and Botanicals Against Blast of Paddy Caused by *Pyriculariaoryzae*,International Journal of Chemical Studies. 5(1):314-318.
26. Kumar S., Lal A., Kumar N., Jaiswal S., Kumar H., Kumar A., Kumar M.,2017. Effect of Bio Control Agents and Botanicals Against Blast of

Paddy Caused by *Pyriculariaoryzae*. International Journal of chemical Studies 5(1):314-318.

27. Magar P.B., Acharya B., Pandey B., 2015. Use of Chemical Fungicides for the Management of Rice Blast (*Pyriculariagrisea*) Disease at Jyotinagar, Chitwan, Nepal. International Journal of Applied Science and Biotechnology, 3(3):474-478.
28. Mandal D.K., Mandal C., Raja P., Goswami S. N. 2010. Identification of Suitable Areas for Aerobic Rice Cultivation in the Humid Tropics of Eastern India. Current Science, 99:227-231.
29. Massey T.E., Stewart R.K., Daniels J., Liu L., 1995. Biochemical and Molecular Aspect of Mammalian Susceptibility to Aflatoxin B1 Carcinogenicity. Proc SocExp Bio Med, 208:231-27.
30. Nagarajan S., 1988. Epidemiology and Loss of Rice, Wheat and Pearl millet Crops due to Diseases. In; International Symposium on Crop Losses and Diseases Outbreaks in Tropics and Control Measures. Tropical Agriculture Research Centre, Japan, 209.
31. Nakamura M., Ishibashi K., 1958. New Antibiotic Ophiobolin, Produced by *Ophiobolusmiyabeanus*. Nippon Nogiekagakukasihi, 32:739-744.
32. Orsenigo M., 1956. Produzione Di TossineDa Parte De *Heliminthosporiumoryzae* Breda de Haan. Pare I. Ann. Sper. Agric. N. S. 10:1745-1762.
33. Orsenigo M., 1957. Estrazione e PurificazionedellaCochliobolin, unatossinoprodotta da *Helminthosporiumoryzae*. Phytopathol. Z. 29:189-196.
34. Ou S.H., 1972. Studies on Stable Resistance to Rice Blast Disease. In : Rice Breeding, Manila. International rice Research Institute, 227-237.
35. Ou S.H., 1985. Rice Diseases: International Rice Research Institute, Manila, Philippines.
36. Paadmanabhan S.Y., 1973. Great Bengal Famine, Ann. Rev. Phvtopath. 11:11-24.
37. Padmanabhan S.Y., 1965. Studies on Forecasting Outbreaks of Blast Disease of Rice. In: Influence of Meteorological Factors on Blast Incidence at Cuttack. Proceedings of the Indian Academy of Sciences, 117-129.
38. Padmanabhan Y.S. 1965. Physiologic Specialization of *Pyriculariaoryzae* Cav. The Causal Organism Blast of Rice Current Science. 34:307-308.
39. Padovani L., Capri E., Padovani C., Puglisi E., Trevisan M., 2006. Monitoring Triciclazole Residues in Rice Paddy Watersheds. Chemosphere,62(2):303- 314.

40. Sesma A., Osbourn A.E., 2004. The Rice leaf Blast Pathogen Undergoes Developmental Processes Typical of Root Infecting Fungi. Nature, 431:582-586.
41. Sester M., Raveloson H., Tharreau D.,Dusserre J., 2014. Conservation Agriculture Cropping System to Limit Blast Disease in Upland Rainfed Rice. Plant Pathology,63:373-381.
42. Shahjahan A.K.M., 1994. Practical Approaches to Rice Blast Management in Tropical Monsoon Ecosystems, Rice Blast Disease. IRRI, Philippines. International Rice Research Institute, 465-488.
43. Shyamala L., Sivakumaar P.K., 2012. Antifungal Activity of Rhizo bacteria Isolated from Rice Rhizosphere Soil Against Rice Blast Fungus *Pyricularia oryzae*, Department of Microbiology, Annamalai University, India.
44. Steyn P.S., RabieC., 1976.Characterization of Magnesium and Calcium Tenuazonate from Phomasorghina. J. Phytochem. 15:1977-1979.
45. Suriya Rao A,V., Nayak P., 2007. Spatial Distribution of Rice Blast Disease Under Natural Field Epidemics. Research Journal of Agriculture and Biological Sciences, 3(6): 615-620.
46. Thomas C. S., Skinner P.W., Fox A.D., Greer C.A., Gubler W.D., 2002. Utilization of GIS/GPS, Based Information Technology in Commercial Crop Decision. 34(3):200-206.
47. Umetsu N., Kaji J., Tamari K., 1972. Investigation on the Toxin Production by Several Blast Fungus Strains and Isolation of Tenuazonic Acid as a Novel Toxin. Agri. Biol. Chem. 36: 859-866.
48. Yun C.S., Motoyamal T., Osada H., 2015. Biosynthesis of the Mycotoxin Tenuazonic Acid by a Fungal NRPS-PKS Hybrid Enzyme Nature Communications, 6(8758):1-9.
49. Zeigler R.S., Leong S.A., Teng P., 1994. Rice blast disease: International Rice Research Institute, Manila, Philippines.