

## Seasonal Occurrence of Diseases on Oyster Mushroom (*Pleurotus* spp.) in new Alluvial Region of West Bengal

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**ABSTRACT:** Oyster mushroom (*Pleurotus* spp.) offers an important opportunity for use as cheap and nutritious food for the human being. When growing oyster mushrooms, a variety of harmful mould fungi are found in the substrate and thereby adversely affecting spawn run, causing huge loss in commercial mushroom production. In the present investigation, attempts were made to identify different competitor moulds and their occurrence in relation to weather variables under the Gangetic alluvial region of West Bengal. One main disease (Green mould) and three minor diseases (Black mould - *Mucor* spp., Inky cap - *Coprinus* spp., and Yellow mould) of oyster mushroom (*Pleurotus ostreatus*) were noticed during the period of survey (2017-18). Black mould and Inky cap infestations were noticed during the monsoon and late monsoon periods. However Green mould disease was initiated during the last week of October and peak disease incidence was recorded during December- January. The incidence of green mould disease was significantly negatively correlated with T. max ( $r = -0.938^{**}$ ), T. min ( $r = -0.868^{**}$ ), average temperature ( $-0.926^{**}$ ). Rainfall, minimum and maximum relative humidity, showed a non-significant negative correlation with green mould disease incidence. Stepwise multiple regression equation revealed that maximum temperature was found to be an important predictor for green mould disease development. Black mould disease was significantly positively correlated with T.min ( $r = 0.696^*$ ), RH.max ( $r = 0.679^*$ ), RH.min ( $r = 0.807^{**}$ ), average RH ( $0.797^*$ ) and rainfall ( $r = 0.794^*$ ) whereas, maximum and average temperature showed a non-significant positive correlation with Black mould disease incidence.

**Keywords:** Green mould, yellow mould, black mould, inky cap disease, mushroom, weather variables.

### INTRODUCTION

The edible oyster mushroom, (*Pleurotus* spp.) is a member of the Basidiomycota fungi group Edible fungi are a source of cheap and nutritious food for human beings. The most commonly cultivated edible mushrooms in India are button mushrooms (*Agaricus bisporus*), oyster (*Pleurotus* spp.), Milky mushroom (*Calocybe* spp.) and Paddy straw mushroom (*Volvariella* spp.). These mushrooms grow well in the different agroecological zones of the country. The future of the mushroom industry in India will depend on how fresh mushrooms are marketed (Karthick *et al.*, 2017). Mushrooms used as food are fruit bodies of macrofungi, which can be edible or poisonous and non-edible. The high content of quality protein, vitamins and fibre makes mushrooms an ideal food item that occupies a place between meat and vegetables from a nutritional point of view. They also contain a good amount of amino acids such as leucine, isoleucine, threonine, tyrosine and phenylalanine (Manikandan, 2011).

*Pleurotus ostreatus*, commonly known as oyster mushroom, exhibits a remarkable ability to thrive on various agricultural by-products and industrial wastes, as observed in the study conducted by Pani *et al.* (1997). Despite its adaptability to different

substrates, pasteurized straw remains the most prevalent medium for cultivating this mushroom species. The increasing popularity of oyster mushrooms can be attributed to their cultivation simplicity, potential for high yields, and excellent nutritional content (Banik and Nandi 2004; Gregori *et al.*, 2007).

Mushrooms, like other crops, are vulnerable to a wide range of both living (biotic) and non-living (abiotic) factors that can negatively impact their growth and yield (Sharma *et al.*, 2007). Biotic agents such as fungi, bacteria, viruses, nematodes, insects, and mites pose significant threats, leading to substantial damage and losses. Common competitor moulds and pathogenic fungi found mainly in compost include olive green mould (*Chaetomium olivaceum* and other species), ink caps (*Coprinus* spp.), green moulds (*Aspergillus* spp., *Penicillium* spp., and *Trichoderma* spp.), black moulds (*Mucor* spp., *Rhizopus* spp.), as well as other varieties like *Myriococcum praecox*, *Sporotrichum* spp., *Sepedonium* spp., *Fusarium* spp., *Cephalosporium* spp., *Gliocaldium* spp., and *Papulospora* spp. In West Bengal, microbial contamination of the oyster mushroom bed is one of the main obstacles to higher yield (Biswas *et al.*, 1997; Sharma *et al.*, 2013). According to studies

conducted by various researchers (Hermosa *et al.*, 1999; Mamoun *et al.*, 2000; Neelam *et al.*, 2014), the main contaminants of *Pleurotus* spp. were *Trichoderma harzianum*, *Aspergillus* spp., *Penicillium* spp., *Moniliastophila*, *Stemonitis* spp. and *Coprinus* spp. Among these contaminants *Trichoderma harzianum* was reported to be the most destructive of these contaminants, strongly competing with the mycelium of *Pleurotus pulmonarius* and *Pleurotus ostreatus in-vitro* and decreasing the production surface by 30 to 50% (Shin, 1987). While, *Aspergillus niger*, *Coprinus* sp, *Penicillium* spp. and *Sclerotium rolfsii* were the most predominant fungal contaminant of mushroom beds of *P. florida* (Biswas and Kuiry 2013). In the mushroom house, on oyster mushroom growers mostly faced green mould disease problems that caused more yield losses (Jaklitsch *et al.*, 2006). On oyster mushroom beds, *Trichoderma*, *Aspergillus*, and *Rhizopus* were the most common Pathogen, and their occurrence was particularly severe in the summer and spring seasons compared to autumn and winter (Jaivel & Marimuthu 2010).

In the mushroom farmhouses of Nadia district of West Bengal, mushroom growers face serious problems of ink caps, black moulds and green mould diseases of oyster mushroom (*Pleurotus ostreatus*) resulting in huge losses of the mushroom crops. Hence, the present study aimed to survey and record on seasonal occurrence of different diseases of oyster mushrooms in a year.

## MATERIALS AND METHODS

### Survey

A periodical survey was carried out in a selected commercial mushroom production farm located at Ghagharchar, Badkulla in Nadia district of West Bengal during 2017-18 to observe and record the seasonal occurrence of different diseases of oyster mushroom (*P. ostreatus*). The poly bag method of oyster mushroom cultivation was adopted in the surveyed farm. During the survey, samples of different moulds like green mould, black mould and yellow mould were collected individually and kept in clean polythene bags with proper labelling and brought into the laboratory for isolation for further studies. The severity and incidence of different diseases were recorded following the under-mentioned disease scale.

The Disease incidence (%) was calculated using the following formula:

$$\text{Disease incidence: } \frac{\text{No.of infected mushroom beds}}{\text{Total no.of mushroom beds assessed}} \times 100$$

### Isolation

From the infected substrates of different characteristic mould symptoms, a single substrate (straw) was separated from others, then surface sterilized with 0.1 per cent sodium hypochlorite solution, rinsed thrice with sterilized distilled water and then transferred aseptically on PDA medium from different mould symptoms in different Petri plates and marked with marker. These Petri plates were incubated at  $25 \pm 2^\circ\text{C}$ .

After 2 to 3 days of incubation, isolates were purified following standard pathological method. The pure culture of isolated fungus was maintained in a slant kept in a refrigerator for identification of the pathogen by microscopic observation.

### Weather based parameter-based disease prediction system against mould disease of mushroom

Observations for disease incidence were recorded once a month from August 2017 to April 2018. Meteorological observatories of AICRP on Agro-meteorology have diligently collected weather data, encompassing crucial information such as maximum and minimum temperatures, morning and afternoon relative humidity levels, sunshine hours, and rainfall. These valuable records serve as essential resources for understanding and analyzing the climatic conditions in the designated areas, B.C.K.V., Nadia, West Bengal. The data of different weather variables were calculated based on the basic weather parameter data. The following weather parameter and variables data were used for the present investigation *viz.*, Temperature max, Temperature min., Temperature average (Tavg), RHmax, RHmin, RHavg, Tavg\*RHavg, Rainfall, Rainfall Transformed (RFtrans.), Tavg\*RFtrans Tavg\*RHavg\*RFtrans. Pearson's Correlations of average monthly weather parameter and variables data with monthly disease incidence was done and stepwise multiple linear regression analysis was done based on the weather parameters and variables as independent variables and monthly disease incidence data as dependent variables.

## RESULTS AND DISCUSSION

The frequent or real-time fixed plot surveys were carried out to record the occurrence of different competitors diseases/competitors' mould of oyster mushrooms, in Badkulla Farm, Nadia district (W.B) during 9 months of cultivation season of oyster mushrooms, (August to April) in the year 2017 to 2018. One main disease (green mould) and three minor diseases (black mould, inky cap and yellow mould) were noticed and there seasonality of infection and periodical disease incidence at monthly intervals were observed and presented in Table 3 and Fig. 3.

### Symptomatological studies of different diseases of mushrooms.

Different diseases have been observed on the mushroom beds (substrate/mushroom). The infected disease samples were brought to the laboratory for isolation and characterization of pathogen (Table 1) and detailed symptomatological studies were undertaken and presented in Table 2.

**Table 1: Collections of mushroom disease samples from mushroom grower field.**

Sr. No.	District	Name of the disease	Identified pathogen
1.	Nadia	Inky cap	<i>Coprinus</i> spp.
2.	Nadia	Black mould	<i>Rhizopus</i> spp.
3.	Nadia	Green mould	<i>Trichoderma</i> spp.
4.	Nadia	Yellow mould	Unidentified fungi



**Fig. 1.** Overview of different diseases of mushroom A. Inky cap disease B. Green mould disease C. Black mould disease D. Yellow mould disease.

**Table 2:** Syntomatological studies of different diseases of mushroom (oyster) observed during field survey

Name of the disease	Name of the pathogen (competitor)	Time of incidence	Symptoms
1. Inky cap	<i>Coprinus</i> spp.	April to September	Ink caps appear in the substrate during the spawn run. They are slender, bell-shaped mushrooms. This weed fungus stalk is narrow and long and its cap is thin. In the mushroom bed, this fungus grows rapidly, and within 24 hours the cap becomes black and decays. The attack of this type of fungus in the mushroom bed inhibits the growth of mushroom mycelium and reduces yield.
2. Black mould	<i>Rhizopus</i> spp.	March to August	<i>Rhizopus</i> , a competitor fungus, is contaminated with black spotting on the surface of the substrate of mushroom beds. The growth of <i>Rhizopus</i> mycelium competes with mushroom mycelia.
3. Green mould	<i>Trichoderma</i> spp.	Last week of October to April	The green mould is a prevalent and highly damaging ailment, posing as one of the most widespread and destructive diseases. A compact and completely white mycelium growth may manifest on the substrate (paddy straw), bearing a striking resemblance to mushroom mycelium. Subsequently, the mycelial mat undergoes a colour transformation to green due to a substantial increase in sporulation, which serves as a distinctive symptom. The mushrooms that grow in the vicinity of this mycelium exhibit a brown colouration, and they may also become cracked and distorted. Under favourable conditions, the whole mushroom mycelium was destroyed and the bed became green.

4.	Yellow mould disease	Unidentified fungi	March to April	This particular fungus creates a corky mycelial layer with a yellow-brown hue at the interface of the substrate (paddy straw), making it challenging to identify during the spawn impregnation process. However, it becomes noticeable once it adopts its stoma-like appearance, leading to a significant hindrance in mushroom production. Ultimately, yellow coloured mycelium appears with spore clod or colony formation on the substrate.
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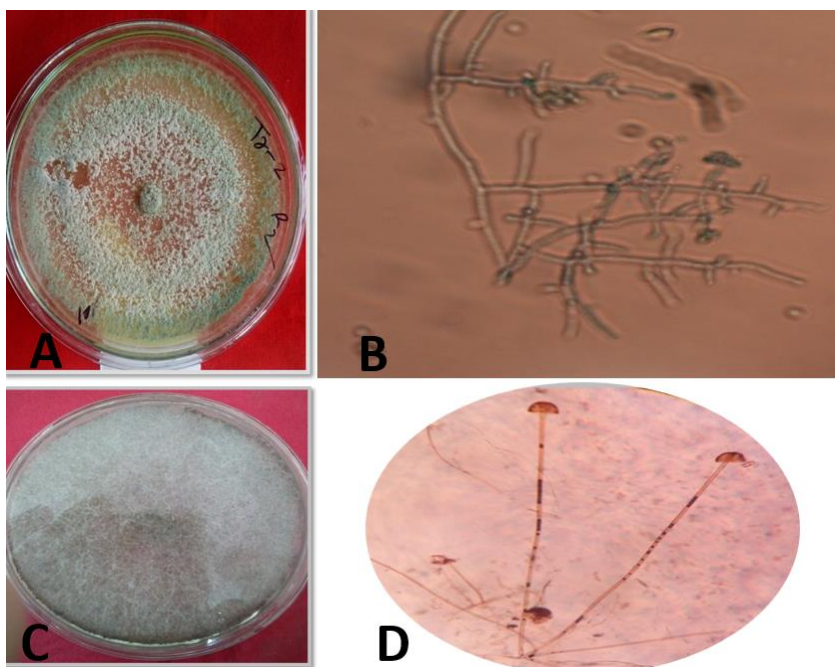
It has been observed that the green mould disease found to peak during the month of December –January

### Identification

#### Identification of the pathogen:

Green mould pathogen grew on the PDA media, colony showed a dark green to dark bluish green colour. Under microscopic observation, the conidiophore is usually a long, infrequently branched, verticillate conidiophore. Phialides are frequently paired, lageniform convergent. The conidial shape was globose to ellipsoidal (Fig. 2. A & B). Based on the cultural characteristic of green mould symptom-producing pathogen (PDA media), microscopic view of mycelium and conidiophores and

conidia it can be concluded that the pathogen is one of the species of *Trichoderma* spp. Black mould pathogen grew on potato dextrose agar media (PDA) started white and cottony, then became heavily speckled with sporangia, then turned brownish-grey to blackish-grey and spread quickly. Sporangiospores, sporangia, and sporangiophores with rhizoid were observed under a light microscope (Fig. 2. C & D). Based on the cultural and morphological characteristics it can be concluded that the pathogen is one of the species of *Rhizopus*. Ink caps was not isolated in media.



**Fig. 2.** Cultural and microscopic photographs of A & B: Green mould pathogen (*Trichoderma* sp.); C & D: Black mould pathogen (*Rhizopus* sp.).

**Table 3: Seasonal incidence of different competitor moulds on oyster mushroom cultivation in Nadia, W.B.**

		Disease incidence (%)			
Occurrence		Coprinus	Black mould	Green mould	Yellow mould
2017	Aug	5.83	4.00	0.00	0.00
	Sep	7.25	4.92	0.00	0.00
	Oct	1.25	1.50	7.08	0.00
	Nov	0.00	0.00	12.92	0.00
	Dec	0.00	0.00	35.42	0.00
2018	Jan	0.00	0.00	28.50	0.42
	Feb	0.00	0.00	11.50	1.75
	Mar	0.00	0.00	3.75	2.08
	Apr	1.83	0.00	0.00	1.25

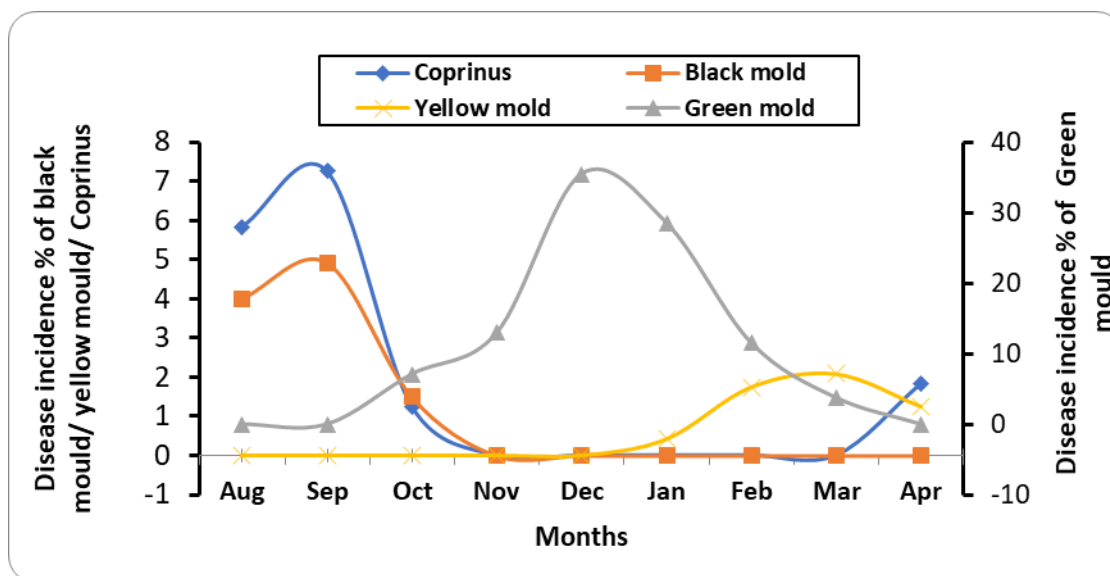


Fig. 3. Seasonal variation of different diseases of oyster mushroom under gangetic alluvial region of West Bengal.

**Seasonal incidence of different competitor moulds on oyster mushroom cultivation in Nadia, W.B.**

The perusal of data is presented in Table 3. Indicated that black mould and inky cap attacks were noticed during the monsoon and late monsoon period that is during the month of August to October. However, Green Mould disease was initiated during the last week of October and peak disease incidence was recorded during December-January. Whereas, yellow mould disease was observed during the dry season (January to April).

In the present investigation among the different competitive moulds *Trichoderma* spp. caused a great threat to oyster mushroom cultivation in gangetic alluvial regions during winter season.

Similarly, during the survey of button mushroom farm in North India, Sharma and Vijoy (1996) reported an incidence of *Trichoderma* spp. Up to 3-50%, and estimated yield loss by the fungi to be 12.5 to 80.8%. On oyster mushroom beds, *Trichoderma*, *Aspergillus*, and *Rhizopus* were the most common Pathogen, and their occurrence was particularly severe in the summer and spring seasons (Jaivel & Marimuthu 2010).

Jandaik and Guleria (1999) collected disease samples from two mushroom farms, and were brought to the

laboratory for isolation and characterization of the pathogen. They reported 5.0 to 46.87% and 6.25 to 50.00 % yield loss due to *Trichoderma viridae* respectively under artificial conditions.

**Influence of different weather variables against green mould**

From the data presented in Table 5, it is revealed that the incidence of green mould disease was significantly negatively correlated with Tmax ( $r = -0.938^{**}$ ), Tmin ( $r = -0.868^{**}$ ), average temperature ( $-0.926^{**}$ ) whereas, rainfall, minimum and maximum relative humidity showed non-significant negative correlation with green mould disease incidence.

Regression analysis (RA) was done considering green mould disease incidence data collected from the commercialized mushroom production farm as dependent variable and weather parameters like temperature (maximum and minimum, average), relative humidity (maximum, minimum, average), total rainfall and the effect of individual as well as combined weather factors for disease development. The stepwise regression technique was used to obtain a model based on only significant variables.

Table 4: Different weather parameters and variables during mushroom growing periods (2017-18).

Months	Tmax	Tmin	Tavg	RHmax	RHmin	RHavg	RF	RFtrans	Tavg*RH avg	Tavg*RFtrans	Tavg*RHavg*RFtran
August	32.9	26.2	29.5	95.4	83.4	89.4	280.0	16.8	2640.2	494.8	44218.3
September	34.2	26.7	30.4	95.7	74.6	85.1	161.1	12.7	2590.6	386.8	32932.3
October	32.1	24.4	28.2	97.5	75.2	86.4	237.7	15.4	2438.0	435.7	37627.3
November	29.3	17.8	23.6	93.1	57.3	75.2	37.0	6.1	1773.1	144.4	10857.8
December	26.0	14.3	20.1	93.7	61.5	77.6	15.4	4.0	1560.9	80.2	6224.0
January	24.4	8.8	16.6	90.9	47.7	69.3	0.0	0.7	1149.9	11.7	813.1
February	30.8	15.7	23.3	90.0	44.4	67.2	0.0	0.7	1563.2	16.4	1105.3
March	35.1	21.0	28.1	89.5	40.2	64.8	1.5	1.4	1817.9	39.7	2570.9
April	35.3	23.5	29.4	89.5	54.0	71.8	51.5	7.2	2111.4	212.2	15225.3

However, considering all the weather variables as predictors to explain key weather factors which influence the disease incidence, the stepwise multiple regression equation revealed that maximum temperature was found to be an important variable for green mould disease development. The coefficient of determination was highly significant and the model fitted well for all the weather data. Average temperature was found to be an important predictor and could able to explain the variation of green mould disease incidence by more than 86 % (Table 6). Different workers recorded similar observations on the influence of temperature on green

mould disease incidence in the different mushroom-pathogen systems. According to the results of the current experiment, the ideal temperature range for the occurrence of green mould disease was between 20 and 25°C. Woo *et al.* (2004) investigated the impact of temperature on the mycelial growth of isolates of green mould and other *P. ostreatus* species. The ideal temperature for *Pleurotus* growth was 28°C, whereas *Trichoderma* could grow well throughout a larger range (20–28°C) and outgrew *Pleurotus* three times faster at 25°C.

**Table 5: Pearson Correlation study of green mould disease incidence with weather variables.**

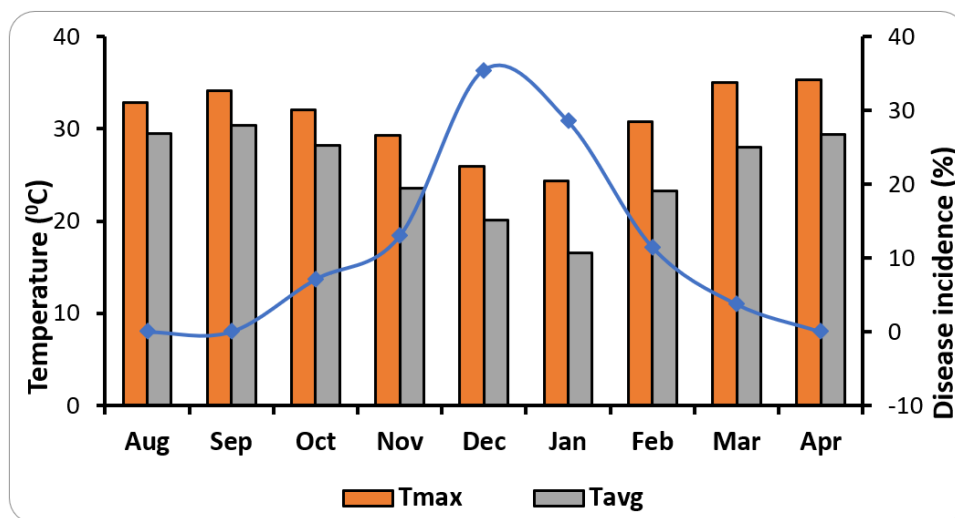
Parameters	Green mould		Parameters	Black mould	
	Correlation coefficient	P value		Correlation coefficient	P value
Green mould	1.000		Black mould	1.000	
Tmax	-0.938**	0.000	Tmax	0.387	0.303
Tmin	-0.868**	0.002	Tmin	0.696*	0.037
Tavg	-0.926**	0.000	Tavg	0.595	0.091
Rhmax	-0.101	0.796	Rhmax	0.679*	0.044
Rhmin	-0.294	0.443	Rhmin	0.807**	0.008
Rhavg	-0.265	0.490	Rhavg	0.797*	0.010
RF	-0.521	0.150	RF	0.794*	0.011
Rftrans	-0.542	0.132	Rftrans	0.786*	0.012
Tavg*Rhavg	-0.774*	0.014	Tavg*Rhavg	0.810**	0.008
Tavg*Rftrans	-0.590	0.095	Tavg*Rftrans	0.818**	0.007
Tavg*Rhavg*Rftran	-0.569	0.110	Tavg*Rhavg*Rftran	0.832**	0.005

Tmax.: Temperature Maximum, Tmin.: Temperature Minimum, Tavg.: Temperature average, Rhmax.: Relative humidity maximum, Rhmin: Relative humidity Minimum, RHavg.: Relative humidity average, RF.: Rainfall.

#### Influence of different weather variables against black mould disease

From the data presented in Table 5, it is revealed that the incidence of black mould disease was significantly positively correlated with Tmin ( $r = 0.696^*$ ), RHmax ( $r = 0.679^*$ ), RHmin ( $r = 0.807^{**}$ ), average RH ( $0.797^*$ ) and rainfall ( $r = 0.794^*$ ) whereas, maximum and average temperature showed a non-significant positive correlation with black mould disease incidence.

Regression analysis (RA) was done considering black mould disease incidence data collected from the commercialized mushroom production farm as dependent variable and weather parameters like temperature (maximum and minimum, average), relative humidity (maximum, minimum, average), total rainfall and the effect of individual as well as combined weather factors for disease development. The stepwise regression technique was used to obtain a model based on only significant variables.



**Fig. 4.** Relationship between Tmax, Tavg with Green mould disease incidence.

**Table 6: Regression equation prediction model of Green mould, Coprinus, Black mould and Yellow mould disease.**

$Y(GM) = 107.46 - 3.1(T_{max})^{**}$ $R^2 = 0.883$ $R^2_{adj} = 0.866$
$Y(Cop) = -6.82 + 0.004(T_{avg} * R_{havg})$ $R^2 = 0.662$ ; $R^2_{adj} = 0.613$
$Y(BM) = -0.452 + .0000955(T_{avg} * R_{havg} * R_{frans})$ $R^2 = 0.692$ ; $R^2_{adj} = 0.648$
$Y(YM) = 22.45 - 0.235(R_{hmax})$

However, considering all the weather variables as predictors to explain key weather factors which influence the disease incidence, the stepwise multiple regression equation revealed that the combined weather variable i.e.  $T_{avg} * R_{havg} * R_f$  transformed was found to be an important variable for black mould disease development. The coefficient of determination was highly significant and the model fitted well for all the weather data. Combined weather variable i.e.  $T_{avg} * R_{havg} * R_f$  transformed was found to be an important predictor and could able to explain the variation of black mould disease incidence by more than 64 % (Table 6).

## CONCLUSIONS

Mushrooms are healthy food for all age groups. Mushroom production is a lucrative avenue with less investments. Among other stress factors that hinder mushroom productivity, diseases cause substantial loss of yield. In the commercial mushroom farm (*P. ostreatus*) of Ghagharchar, Badkulla in Nadia district green mould was the main disease with as high as 35.42% disease incidence followed by black mould, inky cap and yellow mould with 4.92%, 7.25%, and 2.08% respectively. Green Mould disease was initiated during the last week of October and peak disease incidence was recorded during December-January. Whereas, yellow mould disease was observed during the dry season between January to April. Average temperature between 20-25°C was found to be an important predictor and could able to explain the variation of green mould disease incidence by more than 86 %. Combined weather variable i.e.  $T_{avg} * R_{havg} * R_f$  transformed was found to be an important predictor and could able to explain the variation of black mould disease incidence by more than 64 %. If the weather parameters are controlled and maintained at not favorable proportions for the mushroom disease incitants, the disease incidence could be kept at a minimum and yield loss could be managed. Round the year successful mushroom production provides nutritional security and improved livelihood for the growers.

## FUTURE SCOPE

Disease prediction model is a very effective tool for predicting possible impacts of climatic change on disease onset and spread. Disease prediction models are useful for solving various practical problems in present day sustainable agriculture. Monitoring and characterization of pathogen population in mushroom growing region of West Bengal, India. Effect of epidemiological factors associated *Trichoderma* spp on mushroom maintaining of proper temperature for

mushroom cultivation for inhibiting this disease. Identification of important strain of *pleuratus* which are tolerance against *Trichoderma*.

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**Conflict of Interest.** None.

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