Survey, collection and In-vitro study of the stemphylium blight disease in onion caused by *Stemphylium vasicarium*

ABSTRACT

Onion (*Allium cepa L.*) is grown all over world and is a favorite vegetable in India. It is relished mostly as salad and Indian cuisine is incomplete without onion. The survey was done during Kharif 2022 and Rabi, 2022-23 at different taluka of Nashik District. At 75 days after transplanting the highest intensity of stemphylium blight (19.2%) was recorded in taluka Deola. The leaves from the affected plant of different age showing the characteristic symptoms of stemphylium blight was brought to the laboratory in polythene bags for critical examination and presence of the pathogen responsible for symptoms production. The best growth of the fungus on solid medium was recorded (86.0mm) in Potato Dextrose Agar medium while, in case of liquid medium the maximum quantitative growth (793.33mg) was recorded in Richard's medium after 7 days of inoculation. Optimum temperature for the growth of the fungus was 28 °C.

INTRODUCTION

Onion (*Allium cepa*) a bulbous, biennial herb, is one of the most important vegetable crop grown throughout India. Among the various reasons, diseases is important constraints in onion production. The crop is attacked by many diseases, which cause yield losses and also result in deteriorating the quality and export potential. Stemphylium blight caused by *Stemphylium vesicarium* Simmons is one such disease, which was not a major economic threat earlier, but has become a serious problem throughout the country since recent past. Surveys conducted by NHRDF indicated that Stemphylium blight was more severe in the winter/summer than in the rainy season with 1.3 to 100 % incidence (Gupta *et al*., 1994) and sometimes may even cause 100 % crop losses (Singh *et al*., 1992).

Key words: Onion,Disease, Stemphylium blight, Medium, Intensity.

MATERIAL AND METHODS

1- Survey and collection of the stemphylium blight disease plant materials, and recording severity and symptomatology of stemphylium blight under natural condition.

The survey was done in onion crop for the occurrence of stemphylium blight disease in six Taluka namely Niphad, Chandwad, Kalwan, Yeola, Deola and and Satana of Nashik district. Regular and constant observation of onion crops grown at different locations at farmer's fields of Nashik district was made during *Kharif* and *Rabi* crop season of 2022-23.The leaves from the affected plant of different age showing the characteristic symptoms of stemphylium blight was brought to the laboratory in polythene bags for critical examination and presence of the pathogen responsible for symptoms production. The careful examination was made by teasing diseased material and temporary mounts prepared under the microscope. Some better specimens was

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selected dried, pressed well and put in to herbarium sheet and some wet preservation was also made in F.A.A. (Formaline Acetic acid Alcohol) for further references.

Note: The survey was done twice a season at 45 days after transplanting and 75 days after transplanting .The disease severity in terms of Percent Disease Index (PDI) formula given below. For calculating PDI, at least 10 plants randomly in each field should be scored using disease rating scale given below. Based on the rating 10 plants the PDI value should be calculated using formula given belo

Disease Rating Scale:

List 1 :Grading Scale for Disease Intensity

Grade	Disease Intensity (%)
0	No disease
1	1-10
2	11-20
3	21-30
4	31-50
5	51-100

Percent Disease Index (PDI = Sum of all disease ratings X 100

Total number of observed leaves X maximum rating value (5)

Percent Disease Incidence = Infected plants x 100

Total no. of plants

2- Isolation, purification, identification and Koch's postulates.

The experiment was conducted at Plant Pathology Laboratory of Regional Research Station, National Horticultural Research and Development Foundation Nasik, Maharashtra. The stemphylium blight showing initial and quite distinct characteristic symptoms on different onion varieties selected for isolation of the pathogen in culture. The affected leaves first thoroughly washed in tap water in order to remove the dust particles and surface contaminates. Leaves were cut subsequently in small pieces (5mm) along with some healthy tissues with the help of sterilized blade. The pieces was surface disinfected in 0.01 % mercuric chloride solution for 30-

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40 seconds and thereafter was thoroughly washed in 2-3 changes of sterilized water to remove the traces of mercuric chloride. The excess moisture then removed by folding the pieces in between the two folds of sterilized blotting paper under aseptic conditions in the inoculation chamber. These sterilized pieces were transferred in to sterilized petridish of Potato Dextrose Agar medium in the inoculation chamber. Four to five-pieces was placed aseptically in each petridishes at equal distance and incubated at room temperature (25-28°C ± 1°C). The petridishes used for isolation was already sterilized in a hot air oven at 160°C for two hours and poured with Potato Dextrose Agar medium which was sterilized in an autoclave at 15 lbs. presser for 20 minutes. The inoculated petridishes kept at room temperature (25-28°C ± 1°C) and after about 24 hours of incubation, the whitish mycelium growth appeared around the placed pieces in the petridishes. In some petridishes growth took place in thirty hours while in the remaining ones, fungal growth was observed after two days of incubation. Immediately after the appearance of growth from inoculated pieces, small pieces of, mycelial growth along with medium was transferred aseptically in the sterilized culture tube slants. After transferring to slants, microscopic examination of the growth was also done to ensure that the growth is that of *Stemphylium Sp,* after the growth of the pathogen in slants, they was reexamined microscopically. Those showing sporulation and conidia of *Stemphylium vasicarium*was selected for single spore isolation to obtain genetically homozygous culture. Single spore isolation technique was employed. Once the growth was visible repeated transfers was made aseptically and the culture thus obtained was kept under refrigerated conditions for use as and when required.

Pathogenicity Test:-

The pathogencity of the isolated fungus from the affected parts of onion was made on" healthy leaves in order to establish the pathogenic nature of the fungus. The pathogenicity of the Organism was tested according to "Koch's Pastulate". The plants for this purpose were raised in sterilized pots of 30-cm size. The leaves of 15 days old plants were thoroughly washed with sterilized water. Some of the leaves were slightly injured with the help of sterilized needle while a few others were kept uninjured. The leaves were inoculated with spore suspension of the fungus under study by spraying of the inoculum with the help of an atomizer. The suspension was prepared by mixing of sterilized water in the culture medium containing the mycelium mat and spores. After inoculation the potted plants were kept under moist chamber for 48 hours by covering them under polythene bags. This was done to provide optimum humidity to the pathogen for causing infection. Some of the plants treated in the above manner were left un-inoculated to serve as control. The plants were removed after 48 hours to glass house benches and were watched for the appearance of the disease and its development. The development of the disease symptoms was regularly observed and recorded. From the above artificially produced of stemphylium blight symptoms, the pathogen were re-isolated to prove the subsequent steps of Koch's postulates.

3- Growth of the fungus on different culture media. Morphological and cultural characters of the pathogen leading to its identification

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The temporary slides were prepared in cotton blue from one-week-old culture. Themorphological characters of the fungus were recorded aftergrowing it on Potato Dextrose Agar medium inpetridishes. The inoculated petri-plates were incubated for7 days at 28 ± 1°C, the following observation was recorded.

[A] Colony:-

(i) Colour /pigmentation of the colonies

(ii) Types/patterns of the growth

(iii) Sporulation density

[BJ Mycelium:-

(i) Colour of the hyphae

(ii) Type of branching and septation

(iii) Measurement of hyphae

[C] Conidiophore:-

(i) Colour

(ii) Shape and size

(iii) Septation

(iv) Branching

[D] Conidia:-

(i) Colour

(ii) Shape

(iii) Arrangement of conidia

(iv) Size

(v) Septation (No. of transverse and longitudinal septa)

[E] Beak:-

(i) Colour

(ii) Shape

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(iii) Size

(iv) Septation

Cultural Studies of the Pathogen:

Effect of different media:

Present study, the following natural, semi synthetic and synthetic solid and liquid media were used.

[A] Natural Media:

(i) Oat meal agar medium:-

Oat meal	50.00 g.
Agar - agar	20.00 g.
Distilled water	1000.00 ml.

(ii) Onion seed decoction medium:-

Onion seed	100.00 g.
Agar - agar	20.00 g.
Distilled water	1000.00 ml.

[B] Semi- Synthetic Media:-

(iii) Malt extract medium:

Malt extract	30.00 g.
Agar- agar	20.00 g.
Distilled water	1000.00 ml.

(iv) Potato Dextrose Agar medium:-

Peeled potato-	200.00 g.
Agar – Agar-	20.00 g.
Dextrose-	20.00 g.
Distilled water	1000.00 ml.

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[C] Synthetic Media:-

(v) Corn meal agar medium:-

Corn Meal	50.00 g.
Agar – agar	20.00 g.
Distilled water	1000.00 ml.

(vi) Kirchaff's medium:-

Potassium dihydrogen phosphate (KH2 PO4)	1.00 g
Magnessium sulphate (MgS04-7H20)	0.25 g.
Aspargine	1.00 g.
Agar - agar	20.00 g.
Distilled water	1000.00 ml.

(vii) Richard's medium:-

Potassium nitrate (KNO3)	10.00 g.
Potassium dihydrogen phosphate (KH2P04)	5.00 g.
Magnessium sulphate (MgS04-H20)	2.50 g.
Ferric chloride (FeCl2)	0.02 g.
Sucrose	50.00 g.
Agar – agar	20.00 g.
Distilled water	1000.00 ml.

(viii) Sabouraud's medium:-

Glucose	40.00 g.
Agar - agar	20.00 g.
Distilled water	1000.00 ml.

(ix) Standard Nutrient medium:-

Potassium phosphate (KPO4)	1.36 g.

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Sodium corbonate (NaC03)	1.06 g.
Magnessium sulphate (MgS04-H20) ,	5.00 g.
Dextrose	5.00 g.
Asparagine	1.00 g.
Agar - agar	20.00 g.
Distilled water	1000.00 ml.

Preparation of media:-

The media was prepared by using the standard microbiological methods described. Whenever the above mentioned media where used in the form of liquid, agar - agar was not added. All the culture media was sterilized in an autoclave at 15 ibs. presser for 20 minutes. The glasswares were cleaned in chromic acid (Potassium dichromate- 60.00g, Sulphuric acid 60.00 ml. and distilled water 100.00 ml.). The glasswares were first washed in running tap water then finally washed in distilled water. After drying the glasswares were sterilized at 160°C for two hours in a hot air oven.

Inoculum:-

One-week-old culture of the pathogen under study was grown on potato Dextrose Agar medium and used as inoculum in all the experiments. Petridishes containing equal quantity of Potato Dextrose Agar media was inoculated with 5mm disc, cut with a sterilized cork borer from the actively growing region of the fungus. Flasks containing liquid media was also inoculated aseptically with 5mm, culture disc of the pathogen.

Selection of Basel medium:-

To select the basel medium, the isolate under study was grown on nine different solid and liquid media. For solid media, the mycelium growth and sporulation was taken as criteria of the growth, while in case of liquid media the average dry weight of the fungal colony and extent of sporulation was taken into account.

Measurement of the fungal growth:-

(A) Radial growth:-

For measuring radial growth of the pathogen, 20ml of sterilized agar medium was poured in 9.0-cm. diameter sterilized petridishes. After the medium solidified a 5mm. disc of the fungal growth was cut with the help of sterilized cork-borer and placed at the center of each petri dish. These petridishes was incubated, at 28 ± 1°C up to required incubation period. Each treatment was

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replicated three times. The fungal growth was observed daily and final diameter of the fungal growth was measured manually at the 10th day.

[B] Quantitative growth: -

To measure the amount of fungal dry weight of the pathogen, the fungus was grown in sterilized 50 ml. liquid medium in 150 ml. Erlenmeyer flask. These media was inoculated by equal disk (5mm) from the mother culture cut with sterilized cork-borer. After inoculation in the above manner, these flasks were incubated at 28 ± 1°C for 10 days. Each treatment was replicated three times. The fungal growth was observed daily in each medium and the final data was recorded when the growth in any one flask was completed. The procedure adopted was as follows. The fungal mats from each of the flask was filtrate separately through previously weighed Whatman's filter paper number 41. Later the filter papers along with fungal mats was dried in hot air oven at 60°C for 48 hours, cooled in desiccators containing anhydrous Calcium Chloride for 4 hours and weighed on analytical balance. The actual dry weigh of the fungal mat was calculated by deducting the weight of the filter paper already weighed.

Sporulation:-

For estimation of extent of sporulation, dilution method was followed. In this method, spore suspension was prepared by mixing 5-mm. diameter piece of fungal colony from flasks in 10ml. of the distilled water. After vigorous shaking one drop from each suspension was examined under microscope for counting the number of conidia under low power. The observations recorded was categorized as follows:

List 2 : **Categorization of Sporulation Based on Number of Spores**

No. of spores/microscopic field	Category of sporulation
Nill	Absent
1-5	Poor
6-10	Fair
11-15	Good
16 and above	Excellent

Colour:-

Ridgway's (1992) colour standard and nomenclature was used for determining the colour development in the colonies of the pathogen that developed on media.

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Physiological Studies of the Pathogen:-

In the physiological studies of the pathogen observations was made on temperature requirement and effect of the hydrogen-ion concentrations.

(a) Effect of different temperatures on the growth and sporulation of the fungus

The study was conducted on the best-suited synthetic medium (Richard's medium). The conical flask (150ml.) containing 50 ml. medium was taken and this flask was sterilized at 15ibs. presser for 20 minutes in an autoclave. These sterilized flasks with the medium were inoculated with 7 days old culture of the pathogen in equal quantities (5mm. pieces) made with, help of a sterilized cork borer. This flask was incubated at a different temperature Viz. 5°, 10°, 15°, 20°, 25°, 28°, 30°, 35° and 40°C for 10 days. Each treatment had three replications. After 10 days of incubation, the medium containing mycelial mats was filtered through weighed Whatman's filter paper no.42 and these filter papers with the mycelial mat was dried in the hot air oven at 60°C for 24 hours. The weight was taken separately at different temperature. The net dry weight of the filter paper from the total weight of the each case was deducted; sporulation of the fungus on different media was graded accordingly.

(b) Effect of different hydrogen-ion (pH) concentrations on the growth and sporulation of the pathogen

The best liquid medium (Richard's medium) was used for the study of effect of H-ion concentrations for the growth and sporulation of the fungus. The pH of medium was adjusted to desire level with the help of Phillip's pH meter by using N/10 Hydrochloric acid and sodium hydroxide for lower and higher pH values respectively. The pH-adjusted medium was poured in 150ml. glanic and inoculated. After required incubation period, dry mycelia weight as described earlier was recorded.

RESULT AND DISCUSTION

1- Survey and collection of the stemphylium blight disease plant materials, and recording severity and symptomatology of stemphylium blight under natural condition.

The survey was done in six onion crop fields for the occurrence of stemphylium blight disease in six Talukas *viz.*, Niphad, Chandwad, Kalwan, Yeola, Deola and Satana of District of Nashik at 45 and 75 Days after transplanting during *Kharif*, 2022 and *Rabi* 2022-23 and observed for stemphylium blight disease of onion crop in each season and data recorded during the survey.

***Kharif*, 2022**

At 45 days after transplanting the stemphylium blight incidence ranged from 10.0% to 20.0% with intensity 0.4% to 2.0%. The lowest incidence (10.0%) with intensity (0.4%) was recorded in Taluka Deola, while highest incidence (20.0%) with intensity (2.0%) was recorded in taluka Yeola. At 75 days after transplanting the stemphylium blight disease incidence ranged from 50.0 to 80.0% in different taluka of Nashik district. The lowest incidence (50.0%) with intensity (11.2%) was recorded in taluka Satana while, highest incidence (80.0%) with intensity (14.4%) was recorded in taluka Yeola.

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***Rabi*, 2022-23**

At 45 days after transplanting the stemphylium blight incidence ranged from 5.0% to 10.0% with intensity 0.4% to 1.2%. The lowest incidence (5.0%) with intensity (0.4%) was recorded in Taluka Niphad and Yeola while highest incidence (10.0%) with intensity (1.2%) was recorded in taluka Satana. At 75 days after transplanting the stemphylium blight disease incidence ranged from 85.0 to 100.0% in different talukas of Nashik district. The lowest incidence (85.0%) with intensity (16.0%) was recorded in taluka Satana while, highest incidence (100.0%) with intensity (19.2%) was recorded in taluka Deola.

Table1 Stemphylium blight disease incidence and intensity in different taluka of Nashik district during *Kharif*, 2022

District	Taluka	Stemphylium blight incidence and intensity at 45 DAT		Stemphylium blight incidence and intensity at 75 DAT	
		Incidence%	Intensity%	Incidence%	Intensity %
Nashik	Niphad	10.0	1.8	80.0	14.0
Nashik	Chandwad	15.0	1.2	70.0	12.4
Nashik	Kalwan	15.0	1.6	75.0	12.8
Nashik	Yeola	20.0	2.0	80.0	14.4
Nashik	Deola	10.0	0.4	65.0	11.6
Nashik	Satana	10.0	0.8	50.0	11.2

Table2 Stemphylium blight disease incidence and intensity in different taluka of Nashik district during *Rabi*, 2022-23

District	Taluka	Stemphylium blight incidence and intensity at 45 DAT		Stemphylium blight incidence and intensity at 75 DAT	
		Incidence%	Intensity%	Incidence%	Intensity %
Nashik	Niphad	5.0	0.4	85.0	16.0

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Nashik	Chandwad	10.0	0.8	95.0	18.2
Nashik	Kalwan	10.0	0.8	95.0	18.4
Nashik	Yeola	5.0	0.4	90.0	16.8
Nashik	Deola	10.0	0.8	100.0	19.2
Nashik	Satana	10.0	1.2	100.0	18.8

2- Isolation, purification, identification and Koch's postulates.

The experiment was conducted at Plant Pathology Laboratory of Regional Research Station, National Horticultural Research and Development Foundation Nasik, Maharashtra. The isolations were made from the infected leaf lesions as described under materials and methods. The colonies that developed were purified through single spore isolation technique pure culture was obtained for further study.

Pathogenicity test of the pathogen

From the cultures obtained pathogenicity test were done by following Koch's postulate. The process used for the pathogenicity test is described under "Methods and Materials". After inoculation, the plants were watched 'daily and disease development was recorded. The summarized data are presented in table-3. The results revealed that inoculation of upper leaves resulted in higher disease incidence than the lower one. Provisions of injuries were more congenial for disease development; there was no infection in control. The incidence in inoculated plants ranged from 70 to 90% in upper parts while in lower parts it ranged from 60 to 70%.

Table3 Pathogencity test of *S. vesicarium*

Treatment	**Leaf portion inoculated**	**No of leaves inoculated**		**No of leaves infected**		**Per cent of leaves infected**	
		Un - injured	**Injured**	**Un injured**	**Injured**	**Un - injured**	**Injured**
Inoculated with fungus S. vesicarium	**Upper**	10.0	10.0	7.0	9.0	70.0%	90.0%
	Lower	10.0	10.0	6.0	7.0	60.0%	70.0%

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Control	Upper	10.0	10.0	0.0	0.0	0.0	0.0
	Lower	10.0	10.0	0.0	0.0	0.0	0.0

3- Growth of the fungus on different culture media. Morphological and cultural characters of the pathogen leading to its identification

Morphological characters of the pathogen were studied to establish the identity of the causal agent. The results so obtained are as follows.

Colony characters:

The colony that developed on the medium were amphigenous, diffused, dark olivaceous light brown to dark blackish brown, velvety having abundant sporulation.

Mycelial characters:

Mycelium immersed, hyphae branched, septate, hyaline at first, later brown in colour or olivaceous brown, smooth, 0.7-78pm thick.

Character of the sporophores:

The conidiophores arise singly or in groups. They were straight or flexuous, often geniculate, septate, pale to mid brown and up to 116p long and 4.5-1 Op thick.

Conidial characters:

The conidia that developed in large number were muriform oval usually solitary, straight *or* curved, obclavate, tapering to a beak. The conidia were observed to be mid-golden in colour, smooth or minutely verrucose and measure of (with beak) 100-300pm in length and 13- 18p thick at the broadest part. They had 6-11 transverse and zero to several longitudinal or oblique septa.

Cultural characters of the Pathogen:

The present studies were under taken to find out the minimum, optimum and maximum growth requirements of pathogen in culture with regard to media. The details of the techniques followed in these studies are described under "material and methods".

Effect of different media on the growth of the Pathogen:

1- Fungal growth on solid media

Fungi can be grown on different solid media of known and unknown constituents however, with agrestic growth factors *In-Vitro*. They require some specific media for their best vegetative as

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well as reproductive growth. With these views 9 different natural, synthetic and semisynthetic solid media were evaluated for the growth of the fungus and data were recorded after 7 days incubation at 28 ± 1°C. The results are summarized in table-4a.

Table4a Growth and sporulation of *Stemphylium vesicarium*on different solid media after 7 days of inoculation and incubation at 28 ± 1°C.

Sr. no	**Media**	**Average diameter of fungal colonies (mg.)**	**Sporulation**
1	Oat meal agar medium	52.33	XX
2	Onion seed decoction agar medium	40.0	XX
3	Malt extract agar medium	32.33	X
4	Potato dextrose agar medium	86.0	XXXX
5	Corn meal agar medium	45.0	XX
6	Kirchaff's agar medium	65.66	XXX
7	Richard's agar medium	77.0	XXXX
8	Sabouraud' agar medium	72.0	XXXX
9	Standard nutrient agar medium	26.0	X
	S.Em±	**2.34**	-
	CD@ 5%	**4.92**	-
	CV	**5.20**	-

X = Poor Sporulation

XX = Fair Sporulation

XXX = Good Sporulation

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XXXX = Excellent Sporulation

From the data represented in table-4a and corresponding histogram. It is clear that the best growth of the fungus was obtained in Potato Dextrose Agar medium. The growth increased was obtained. The significantly highestgrowth of (86.0mm) that was recorded on PDA followed by Richard's Agar (77.0mm) and Sabouraud's agar (72.0mm).

2- Fungal growth on liquid media

A quantitative weight of the fungal growth was taken after finding out their dry mycelial growth after seven days of incubation at 28 ± 1°C for each replication separately and presented in table-4b.

Table 4b Fungal dry weight and sporulation of *Stemphylium vesicarium*on different liquid media after seven days of incubation at28 ±1°C.

Sr. no	Media	Average dry mycelium growth (mg.)	Sporulation
1	Oat meal medium	558.0	XXX
2	Onion seed decoction medium	585.0	XX
3	Malt extract medium	506.66	XX
4	Potato dextrose medium	651.33	XXXX
5	Corn meal medium	716.66	XXXX
6	Kirchaff's medium	784.33	XXXX
7	Richard's medium	793.33	XXXX
8	Sabouraud' medium	445.33	XX
9	Standard nutrient medium	394.66	X
	S.Em±	**5.47**	-

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	CD@ 5%	11.50	-
	CV	1.1	-

X = Poor Sporulation

XX = Fair Sporulation

XXX = Good Sporulation

XXXX = Excellent Sporulation

The data presented in table 4b and its Corresponding *histogram,* it was clear that the best growth of fungus was obtained in Richard's medium (793.33mg). The next medium is order of merit of growth was Krichoff's medium followed by corn meal medium, Potato dextrose medium

Selection of basal Medium

The best growth of the fungus was obtained on potato dextrose agar medium in which sporulation was also abundant. A comparative study of the growth of fungus on solid and liquid media shows that on potato dextrose agar, the fungal growth was best among all solid media tested; however in liquid media it supported only moderate growth.

Physiological Studies of the Pathogen:-

(a) Effect of different temperatures on the growth and sporulation of the fungus

The experiment was conducted at Plant Pathology Laboratory of NHRDF, Regional Research Station, Nashik, Maharashtra during 2022-23 to find out the range of temperatures (Min., Max. & Opt.) for the growth of the fungus. On Richard's medium, which proved to be one of the best suited media for the growth of fungus was employed. The fungus was grown in conical flasks (150 ml.), which were incubated at selected temperature for 10 days. As the end of the 10th day dry weight of the fungus was determined in each of the conical flasks separately. The average data are recorded are presented in table-5.

Table 5 Fungal dry weight and sporulation of *Stemphylium vesicarium*on different temperature.

Sr. no	Temperature °C	Average dry weight of	Sporulation

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		fungus(mg.)	
1	5	20.0	XX
2	10	181.0	XX
3	15	329.0	XX
4	20	537.33	XXX
5	25	937.66	XXXX
6	28	975.0	XXXX
7	30	949.66	XXXX
8	35	433.33	X
9	40	17.66	X
	S.Em±	**3.88**	-
	CD@ 5%	**8.16**	-
	CV	**0.98**	-

X = Poor Sporulation

XX = Fair Sporulation

XXX = Good Sporulation

XXXX = Excellent Sporulation

Optimum temperature for the growth of the fungus was 28 °C followed by 30 and 25 °C. The fungus was able to grow at a wide temperature range of 5°C to 40°C. It is also obvious from the results that the excellent sporulation was Obtained at 28°C, good at 20°C, 25°C and 30°C, Fair at 5°C, 10°C and 15°C while the sporulation was poor at 35°C and 40°C. Thus, it is clear from the above findings that 28°C was the optimum temperature for the growth and sporulation of the fungus.

(b) Effects of different hydrogen-ion concentration on the growth and sporulation of the fungus

The experiment was conducted to find out the best pH as well as ranges of pH levels for the growth of *Stemphylium vesicarium.* This study was made on Richard's medium. The effect of

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seven different hydrogen-ion concentrations in terms of dry mycelia weight and sporulation of the fungus was recorded and data have been summarized in table-6.

Table 6 Average mycelial dry weight and sporulation of fungus at different pH levels after 10 days of incubation at 28 ± 1°.

Sr. no	**pH level**	**Average dry mycelial weight (mg.)**	**Sporulation**
1	3	123.33	X
2	4	239.0	XX
3	5	681.66	XXX
4	6	795.66	XXXX
5	7	883.0	XXXX
6	8	768.33	XXX
7	9	258.33	XX
	S.Em±	**5.52**	-
	CD@ 5%	**11.83**	-
	CV	**1.26**	-

X = Poor Sporulation

XX = Fair Sporulation

XXX = Good Sporulation

XXXX = Excellent Sporulation

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It is evident from the result presented is table-6 and its corresponding histogram that the fungus was able to tolerate a wide range of pH from 3.0 to 9. The best growth of fungus was recorded at pH 7.0 and 6 followed by 5 and 8. Excellent sporulation occurred at pH 7.0 and 6.0. There was good sporulation at pH 5.0 and 8.0 and it was fair at pH 4.0, while poor at pH 3 and 9.0.

Impact of different fungicides and bio agents against the stemphylium blight under *In-Vitro* condition.

Eight different fungicides were tested according to the procedure mentioned under "Materials and Methods". The growth of the pathogen was measured and average diameter of colony in each petridish was calculated. The results are recorded in table-7.

Table 7Average colony diameter of *Stemphylium blight in* different fungicides and bio-agent at 28 ± 1°C:-

S. N.	**Treatments**	**Dose in %**	**Average Colony diameter (mm)**
1	Carbendazim12% + Mancozeb 63% WP	0.2	5.66
2	Metalaxyl 4% + Mancozeb 64% WP	0.2	4.66
3	Captan 70% + Hexaconazole 5% WP	0.2	5.33
4	Tebuconazole50% + Trifloxystrobin 25% WG	0.15	2.33
5	Metiram55% + Pyraclostrobin 5%	0.15	3.0
6	*T.viride*	0.5	34.0
7	*Ps. fluorocence*	0.5	39.33
8	*Bacillus subtillis*	0.5	40.33
9	Control		92.33
	S.Em±	**1.17**	-
	CD@ 5%	**2.45**	-
	CV	**5.66**	-

It is apparent from the result presented in table-7 that all the fungicides were superior over control. They inhibited the growth of the pathogen. Tebuconazole 50% + Trifloxystrobin 25% WG, Metiram55% + Pyraclostrobin 5% (2.33mm) and Metalaxyl 4% + Mancozeb 64%

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WP(3.0mm) were most effective against the test pathogen as they almost completely inhibited the growth of pathogen.

Stemphylium bligh caused by *Stemphylium vesicarium*is distinctly one of the most important diseases. Stemphylium blight in the surveys conducted of the farmers fields in Nashik District of Maharashtra Stateadjoiming areas of onion, was observed to be serious problem affecting the crop. Not much particularly the management aspect is known. Therefore, the present investigation was carried out in detain to fill the lacuna in our knowledge and for making effectivestrategies to overcome this melody. Different aspects ofstetmphylium blight of onion were investigated the surveys ofonion crop in the month of September, October,2022 for Kharif season and February and march,2022-23 for Rabi season indifferent growing areas of Nashik District revealed thatstemphylium blight of onion occurred widely and under natural conditions, the average disease intensity varied from 11.2% to 14.0% during kharif 20222, while 16.0% to 19.2 %during rabi,2022-23. Asdescribed in experimental results, the description of disease symptoms was in accordance to documenteddetails by several workers notably by Gupta *et. al.* (2008), Kanno and Kanno *et. al.*(1997), Aveling (1998) and Koike and Koike*et. al.*(1998).Thus, the disease understudy is purple blotch and stemphylium blight. Pandey *et. al.* (2019) reported that antracol fungicide inhibit the growth of *stemphyliumvesicarium* under in vitro condition. Pathogenicity test was carried out to establish thepathogenic behaviour of isolated fungus. As a result ofpathogenicity and Koch's Postulates most of the isolateswere found pathogenic and caused the stemphylium blight ononion. Infection of the crop with insects particularly theThribs resulted in predisposition of the crop and thereforemore severity of the disease. These observations have alsobeen recorded by several others [Datar (1996),Cartwright *et. al.* (1995)].It was found that infection was always higher when in plant were injured. This fact suggested that the injuriespredispose the plants to the fungal attack.The causativefungus under study was found exactly similar to thatdescribed by Angel (1929) and Gupta *et al.* (1983). Inorder to find out the culture medium that supports bestgrowth and sporulation, the fungus was grown on ninedifferent natural, semi-synthetic and synthetic solid media.The causal organism *Stemphylium vesucarium*of onion grew beston potato Dextrose Agar medium followed by Richard'sagar medium, Sabouraud's agar medium, KirchofFs agarmedium, Oat meal agar medium, corn meal agar mediumand seed decoction medium, These findings. are almostsimilar as findings of Hossain *et al.* (1997).In liquid media, best quantitative" growth was foundon Richard's medium followed by KirchofFs medium, cornmeal medium, Potato Dextrose medium, seed decoctionmedium, Oatmeal medium and Malt extract medium. Theexcellent sporulation was observed on Richard's mediumfollowed by potato Dextrose medium and seed decoctionmedium, while good sporulation was observed onKirchofFs medium, corn meal medium and Oatmealmedium. Fair sporulation was observed on Malt extract andSabouraud's medium, while poor sporulation was recordedon standard nutrient medium. Such observations do exit[Suemitsu*et al.* (1990)].Temperature is one of the most important physicalfactors affecting the metabolic activity of fungi. There iscertain range of temperature within, which a particularfungus can grow and this range varies from species tospecies. The present test fungus

Comment [H7]: Not similar as in bibliography

Comment [H8]: Is missing in bibliography

Comment [H9]: Should be 2013

Comment [H10]: tribes

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was grown at ninedifferent temperatures Viz. 5°, 10°, 15°, 20°, 25°, 28°, 30°,35°, and 40°C . Optimum growth was recorded at 28°C and25°C. This finding is almost similar to those of Gupta andPathak (1988).The pathogen grew at a wide range of pH from 3.0 to9.0, with minimum and maximum mycelial dry weight atpH 3.0 and 7.0, respectively. The optimum range for thefungal growth as well as excellent sporulation was at pH6.0-7.0, poor growth and sporulation was observed at pH3.0. Docampo and Conci (1996) have reported similarresults with respect to pH level. The results are similar tothe observation made by Hossain *et al.* (1997) whoreported that *Alternaria porri*grew and sporulation wasbest at a pH 7.0 (neutral).

Comment [H11]: spacing

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