

BIOLOGICAL MANAGEMENT OF STEMPHYLIUM BLIGHT OF ONION

by

Miss. Kagane Swati Ramesh

(Reg. No. 016/221)

A Thesis submitted to the
**MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI – 413 722, DIST. AHMEDNAGAR
MAHARASHTRA, INDIA**

in partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY



**DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL
MICROBIOLOGY**

**POST GRADUATE INSTITUTE
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MAHARASHTRA, INDIA.**

2018

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part
there of has not been submitted
by me or other person to any
other University or Institution
for a Degree or
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Date : / /2018

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CERTIFICATE

This is to certify that the thesis entitled, “**BIOLOGICAL MANAGEMENT OF STEMPHYLIUM BLIGHT OF ONION**” submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri Dist. Ahmednagar (M.S.) in partial fulfillment of the requirement for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY**, embodies the results of a piece of *bona fide* research work carried out by **Miss. KAGANE SWATI RAMESH** under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

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Date : / /2018

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ACKNOWLEDGEMENT

Every successful work indirectly is an output of many people's efforts and even this one is not an exception!

It is my privilege to express my deepest gratitude and enormous indebtedness to my Chairman and Research Guide Dr. C.D. Deokar, Head. Department of the Plant Pathology and Agricultural Microbiology, MPKV, Rahuri for his keen interest, dynamic guidance constant inspiration, constructive criticism and encouragement throughout my research work. Without his insistence and persistence this manuscript would not have seen light of the day. I feel extremely honored for getting a golden opportunity to work under his perpetual motivation where I developed insight as how to carryout scientific research work due to his highly technical and critical methodical approach towards work.

I wish to express my profound sense of gratitude to Dr. A.L. Pharande, Dean, Faculty of Agriculture, M.P.K.V., Rahuri and Dr. R.S. Patil, Associate Dean, Post Graduate Institute, M.P.K.V., Rahuri for providing excellent laboratory facilities and never ending inspiration during the investigation.

I gratefully acknowledge my indebtedness and sincere thanks to the advisory committee member, Dr. K.S. Raghuwanshi, Associate Professor, Department of Plant Pathology and Agril. Microbiology Dr. S.V. Kolase Assistant Professor, AICRP on fruits, MPKV, Rahuri. Dr. V.S.Wani Assistant Professor, Department of statistic, MPKV., Rahuri for precise advice, helpful comments and co-operation throughout this investigation.

I also express my deepest thanks to Dr. C.D. Deokar, Dr. B.G. Barhate, Dr. B. M. Ilhe and Dr. A. M. Nawale for their excellent teaching and persistent help in my academic career.

I am also thankful to Sau. Manisha Nangare, Sau Harishchandre madam, Ms Rupali madam and other staf members of Department of Plant Pathology and Agricultural Microbiology, PGI, Rahuri for their help and co-operation.

I am thankful to my senior for their kind co-operation and suggestions during this investigation.

I am extremely thankful to all my batchmates Ms. Mukta Joshi, Mr. Pankaj Shinde, Shiddesh Dhavale, Ms. Madhuri Udavant, Sachin Sirsat, Ajayshree. T.S., Mr. Vaibhav Uchade, Anand Pal, Amol Sutar, Amol Mali for their excellent company and valuable help.

Really fortunate I am to have a juniors I record my deep sense of appreciation of their cheerful company co-operation during my investigation.

Words are not enough to express my gratitude, love and affection to my loving grandfather Shri. Kishanrao Rajaram kagane(mothebaba), grandmother Smt. Indrabai Kishanrao Kagane (May), father Shri. Ramesh Kishanrao Kagane (Baba), mother Smt. Sakhubai Ramesh Kagane (Aai) also I am great thankful to my sister Miss. Kranti Ramesh Kagane and my brother Mr. Prashant Ramesh Kagane and other relatives for their everlasting love unfailing support, constant inspiration, moral support and encouragement during my life.

I am specially thankful to my loving dearest and my source of happiness Ms. Neha, Kalpana, Swati, Priti didi, Pallavi didi, Abhinay for her constant mental and moral support to me and for the encouragement during my life.

I am grateful to all authors past and present whose literature has been cited.

I would like to express my sincere thanks to the Mahatma Phule Krishi Vidyapeeth, Rahuri. Dist-Ahmednagar for providing me an opportunity to undertake my post graduate studies in this “Institute of Excellence”.

Lastly I thank one and all who have directly and indirectly helped me in course of this investigation and whose name could not appear in this acknowledgement.

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LIST OF ABBREVIATIONS

S.E.	:	Standard error
Spp	:	Species
UV	:	Ultra violet
<i>viz.</i>	:	Videlicet (Namely)
%	:	Per cent
µm	:	Micrometer
/	:	Per
@	:	At the rate of
°C	:	Degree celcius
Abst.	:	Abstract
C.D.	:	Critical difference
cm	:	Centimeter (s)
C .V	:	Coefficient of variation
<i>et al</i>	:	And others (et alli)
t/ha	:	tone per hectore
etc.	:	And so forth (<i>et cetera</i>)
Fig.	:	Figure
G	:	Grams
<i>i.e.</i>	:	That is
mms	:	Millimeter
RBD	:	Randomized Block Design

ABSTRACT

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Research Guide : Dr. C.D. Deokar

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Stemphylium blight is a severe disease of onion in Maharashtra caused by *Stemphylium vesicarium* (Wallr). The organism was isolated from infected plant leaves collected from field of central campus, Mahatma Phule Krishi Vidyapeeth, Rahuri. The infected plants initially exhibited characteristic symptoms of tip necrosis with white flecks that enlarged and produced sunken purple lesions, which were surrounded by a yellow to pale brown border. Later, small light irregular to oval, yellow to brown, water soaked and non-delineated lesions developed on older leaves. Lesions usually turn light brown to tan at the centre and later dark olive brown to black. In view of the commercial importance of the crop and losses caused by disease in the field, the present study was undertaken.

The pathogenicity of the organism isolated was confirmed by undertaking the experiments on healthy onion plants of which the Koch's postulate were fully satisfied. From this it was concluded that fungus *Stemphylium vesicarium* is the potential pathogen of stemphylium blight disease of onion.

In the present study, the aqueous plant extract of ten medicinal plant viz., *Azadirachta indica*, *Lantana camara*, *Ocimum sanctum*, *Pongamia pinnata*, *Aloe vera*, *Ginger officinalis*, *Allium cepa*, *Allium sativum*, *Datura stramonium*, *Parthenium hysterophorus*, and five biocontrol agents *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma hamatum*, *Trichoderma konnigii* and *Pseudomonas fluorescens* were tested against *Stemphylium vesicarium* for, their antifungal activity in *in vitro* condition.

Among these the inhibition of mycelial growth by aqueous extracts of all ten plants and biocontrol agents were studied by employing poisoned food techniques and dual culture technique on PDA media. The *Trichoderma viride* was found significantly superior in inhibiting the growth of *S. vesicarium* followed by *Trichoderma harzianum* over control. Among plant extracts *Allium sativum* and *Aloe vera* inhibited mycelial growth of fungus very effectively.

Further extract of these plants and biocontrol agents, which are significantly superior and biofertilizer were also studied under field condition. In field also with three sprays at 15 days interval it was observed that *T. viride* and *T. harzianum* were superior to control. Among plant extracts *Allium sativum* and *Aloe vera* gave most effective results. Also in respect of biofertilizer, *Azotobactor* and PSB gave effective results over control in per cent disease intensity and per cent disease control.

Among bio control agent *T. viride* and *T. harzianum* at 0.5% showed maximum growth of inhibition. Garlic clove extracts at 10% showed maximum growth inhibition among the plant extracts. Thus, the study indicated that suitable intergration of more efficient ecofriendly treatments like bioagents and botanicals, biofertilizer may provide a better management of the *Stemphylium* blight of onion.

1. INTRODUCTION

Onion a member of *Amaryllidaceae* belongs to genus *Allium*. It (*Allium cepa* L.) is an extremely important vegetable crop not only for consumption but also as the highest foreign exchange earner among the vegetables. The origin of onions remains an enigma, but they are believed to have originated from Central Asia (Vavilov, 1951). Onion takes its name from a city built byonias near the Gulf of suez (B.C.173). In sanskrit it is called as “**palandu**”. However, the highest onion diversity is observed in the eastern Mediterranean region and through Afghanistan, Pakistan and India (Brewster, 2008 and Kik, 2008). About 300 species of *Allium* are known. There are hundreds of varieties of onion grown in the world. According to colour, there are red, white and yellow types. Red and white varieties are grown in India. The red colour of onion due a pigment anthocyanin and yellow colouris due to quaracitin pigment. The most important charecteristics of onion is its pungency, which is due to volatile oil allyl propyl disulphide. The nutritive values of onions vary among varieties and generally it contains moisture, carbohydrates, protein, fibre, minerals and energy. Apart from these, also contains minerals like calcium, potassium, phosphorus, sodium, iron and vitamins like vitamin A, vitamin C, niacin, thiamin and riboflavin.

Onion is one of the most widely used vegetable due to its flavoring and seasoning the food, both at mature and immature bulb stage. Besides, it is being used in the manufacture of soups, ketchups, salad and pickles. Medicinal values of onion are innumerable and is one of the ancient crops being utilized in medicine. Extract of onion is used against snake/scorpion bites. It is also used in the prevention of ‘atherosclerosis’ and ‘coronary heart disease’ as they can inhibit the aggregation of human blood platelets to form the clots, which have the potential for arterial blocking. Known to reduce body sugar and the fat. The bulb is useful as diuretic and heart stimulant. To a lesser extent, it is used by processing industry for dehydration in the form of onion flakes and powder, which are in great demand in the world market. Although, onion is consumed in all the countries of the world, it is cultivated only in some countries. Hence it has export market and export value.

Onion (*Allium cepa* L.) is the most important *Allium* species cultivated worldwide, and it is used as vegetable, salad and spice in the daily diet by large population. Onions have an extensive culinary, dietary, therapeutic, trading, income and employment generation value. The three main seasons of *kharif* (monsoon), late *kharif* and *rabi* (winter) contribute 15, 15 and 70 per cent, respectively, to the total onion production in India. India produced around 5.95 million metric tons of onion from 0.44 million ha in 2004 and is the 2nd largest onion producer in the world after China (FAO, 2004). The per hectare productivity ranges from 7.36 tons to 20.00 tons with a mean yield of 12.78 tons and Maharashtra contributes about 30 per cent of India's total production. Onions are the second most cultivated vegetable crop worldwide after tomatoes (FAOSTATS, 2015). In 2012, the area of onion cultivated worldwide was approximately 4.3 million hectares in over 170 countries, generating \$4.0 billion in revenue. China, India, and the USA are the three highest onion producers. These countries produce more than 50 % of the world's onions (FAOSTATS, 2015). The average yield is 17 tonnes per hectare. India produced around 19.71 million metric tonnes of onion from 1.18 million hectare in 2016-17. With 21.6 million tonnes estimated onion production in the country in 2017-18. India ranks second in the world of onion production and first being china. India export onion mainly to the other countries like Malaysia, Russia, Kuwait, Singapore, Germany and Japan. The important onion producing states are Maharashtra, Gujarat, U.P., Orissa, Karnataka, Tamil Nadu, M.P., Andhra Pradesh and Bihar. Maharashtra ranks first in onion production with a share of 18 per cent, however, in terms of productivity, Gujarat ranks first at 22.9 per cent.

In Maharashtra, though Nasik, Ahmednagar, Pune and Satara are the major onion producing districts, the production is taken in three seasons, i.e. *Kharif* (May-July to Oct-Dec.) Late-*Kharif* (Aug-Sep. to Jan-Mar) and *Rabi* (Oct-Nov. to April-June). Though onion is produced in three seasons, the onions produced in rabi season are only suitable for storage as the variety grown in this season has higher TSS, dry matter and more number of outer dried intact scales. The productivity of onion can be affected by number of factors including diseases. Wherever, diseases play an important role in reduction of production and quality. Seed is a basic and vital input and decides the commercial success of a crop variety.

Onion is attacked by many diseases, which vary from region to region, season to season and variety to variety. Diseases can affect it at production, harvesting, processing and marketing, which lower the quality, reduce the yield, and thereby increase the cost of production, and also, the export potential. Purple blotch and **Stemphylium** blight are the most important diseases in Northern India, which causes considerable losses in seed crops as well as bulb crops. It can cause severe damage, especially to the onion seed crop and losses of about 80 to 85 per cent on the crop by affecting leaves and seed stalk (Tomaz and Lima, 1986).

This disease is Caused by *Stemphylium vesicarium*. This disease is very common in Northern and Eastern India on onion leaves as well as flower stalks. Disease intensity varies from 20 to 90 per cent in seed crop and 5 to 50 per cent in bulb crop. The injuries made by thrips are found to enhance the invasion of the fungus *A. porri*. Keeping in view the problem of purple blotch and Stemphylium blight of onion in Tarai and Bhabar region of Uttarakhand causing enormous losses in seed production.

The causal organism is *Stemphylium vesicarium*. About 60 onion and garlic diseases were described by Schwartz and Mohan (2008), 40 of which were caused by fungi, 14 by bacteria, on by yeasts, six by nematodes, three by viruses, and one by phytoplasma like organisms and 7 miscellaneous disease and disorder. Among these diseases stemphylium blight commonly known as white blotch, caused by *Stemphylium vesicarium*, is an important disease throughout the world (Bose and Som 1986; Meah and Khan, 1987 and Castesllanos-Linares *et al.* 1988).

Stemphylium vesicarium, the causal agent of white blotch of onion is considered as the pathogen that initiate the infection, which facilitates the subsequent infection of *Alternaria porri* causing purple blotch and hence presently the disease is named as purple blotch complex of onion. In India the disease was first recorded by Rao and Pavgi in 1975. The disease has now become serious in Northern parts of the country. About 90% losses in seed yield were recorded in India (Anonymous, 1982). Severity of disease was also reported from other parts of the world, viz. Europe, Africa, North and West America (Ellis, 1971).

Wet and warm conditions favours the disease spreading. Under tropical conditions, the disease is a limiting factor for yield of onion. Onion seed production is

severely affected because the disease causes breaking of floral stalks (Munoz *et al.*, 1984). The first symptoms of disease appear on the leaves. The symptoms develop in the middle of leaf as small, yellow to orange streaks which soon develop into elongated, spindle shaped to ovate elongate diffusate spots surrounded by characteristics pinkish margin. These spots turn gray at the center, later brown to dark olive brown with the development of conidiophores and conidia of the pathogen (Miller *et al.*, 1978). The infection is usually confined to the leaves and do not extend down to the scales of the bulb. Similar symptoms are developed on the inflorescence stalk of the onion.

The symptoms develop only on the dorsal side of the leaf / stalk. When seed stalks are infected, the seed do not develop and remain shriveled. The affected leaves and stalks fall down and die. At the harvest, the affected bulbs may show decay. Thus, the disease in quality of bulbs and seed. Yield losses varies from 80 to 85%. However, the indiscriminate use of chemicals is hazardous to microbial population, living beings and it would also lead to a serious soil and water pollution. Onion bulbs are also used for direct consumption.

Spraying of fungicides will cause residual effects. Looking to the severity of the disease, there is need to study the disease in a systemic way. Hence, to find out alternative mean to chemical fungicides, botanical pesticides or bio agents and biofertilizer should be used. The present investigation aims to evaluate the effectiveness of certain plant extracts against the pathogen of Stemphylium blight diseases of onion plants (*S.vesicarium*) in vitro and under field conditions. With a view to identify effective bio agents against *S.vesicarium* the causal organism of Stemphylium blight of onion, present study was proposed with following objectives

1. To isolate the organism associated with *Stemphylium* blight of onion
2. To identify associated pathogen and prove pathogenicity test
3. To see the effects of plant extract for management of disease
4. To see the effect of biocontrol agents for management of disease
5. To see the effects of biofertilizer for management of disease

2. REVIEW OF LITERATURE

The status of research on *Stemphylium vesicarium* (Wallr.) Simmons with regard to history, occurrence and distribution, symptomatology, pathogenicity tests, management strategies are discussed in the following headings below

2.1 History

Stemphylium vesicarium as the causal organism of stemphylium leaf blight of onion was first reported by Simmons (1969). Later on, it was supported by other workers in India and abroad (Rao and Pavgi, 1975; Miller *et al.*, 1978). Wallroth (1833) first reported the association of Stemphylium with asparagus (*Asparagus officinalis* L.) and named the genus Stemphylium. Wiltshire (1938) proposed the original and modern concept of Stemphylium. A leaf spot disease of onion caused by *S. vesicarium* was first reported from Nasik district of Maharashtra (Patil and Patil, 1991).

2.2 Occurrence of the disease

Su (1936) reported *Stemphylium* blight of onion caused by *Stemphylium vesicarium*, and its perfect state *Pleospora alliis* *Macrosporium parasiticum* (*Pleospora herbarum*) from Barma. stemphylium blight of onion was first recorded by Rao and Pavgi (1975) in India. stated about stemphylium blight caused by *stemphylium vesicarium*, a serious pathogen, which confirmed to leaves and fluorescence stalk.

Miller *et al.* (1978) reported on *Stemphylium* blight of onion in South Texas, a previously unrecognized disease of onion cause significant damage during 1976, both alone and with *Alternaria porri*.

Wu (1979) surveyed on the seed-borne diseases of vegetables. Results of the survey on onion showed that *Alternaria porri* and *Stemphylium botryosum* (*Pleospora herbarum*) reduced germination of onion seeds.

Inoculations with *Stemphylium vesicarium* on the short day onion cultivars, White Granex, Ben shamen and New Maxico Yellow Grano produced lesions identical to those in the field.

Miller (1983) reported that measurements of infected leaves were taken weekly from bulb initiation to bulb maturity of onion.

S. vesicarium has been reported on several hosts from several countries as on Lucerne from South Africa (Lamprecht *et al.*, 1984), on asparagus from Washington state (Johnson 1987) and South Africa (Thompson and Uys, 1992), on pears from Italy (Brunelli and Penti 1989) and Spain (Baroja, 1999), on coconut from Southern Oman (Hammouda, 1991), on onion from Portugal (Tomaz and Lima, 1986), Spain (Basallote *et al.*, 1999) and South Texas (Miller *et al.*, 1978), on *Allium* spp. (Cho and Yu, 1998) from Korea and on garlic from South Africa (Aveling and Naude, 1992), Brazil (Boiteux *et al.*, 1994). Spain (Basallote *et al.*, 1999) and Australia (Suheri and Price 2000).

The causal agent of severe foliar blight on garlic (*Allium sativum*) and on onion (*Allium cepa* L.) at the Rangel and Sucre municipalities of Merida State, Venezuela, was identified as *S. vesicarium* and this was the first report of *S. vesicarium* causing severe leaf blight disease on garlic and onion in Venezuela (Cedeno *et al.*, 2003).

In India, *S. vesicarium* was reported on onion from Maharashtra (Patil and Patil, 1992) and from Bihar (Sinha *et al.*, 1996). Sinha *et al.* (1998) have observed the anamorph *S. vesicarium* and teleomorph *Pleospora* spp. on garlic as a new record for India. The incidence of leaf blight on garlic has also been reported from Himachal Pradesh (Sugha and Kumar, 2005). Shubana *et al.* (2008) have observed for the first time the Stemphylium blight of onion pathogen *S. vesicarium* (Wallr.) E. Simmons; perfect state *Pleospora allii* (Pers. ex. Fr.) Rabenh.) from Jammu and Kashmir.

2.3 Symptomatology

The SLB first appears as elongated, small spindle shaped white flecks, which enlarge and produce sunken purple lesions often surrounded by a yellow to pale brown border. These lesions cause extensive necrosis, resulting in premature drying of leaves and cessation of bulb development (Hill, 1995; Basallote-Ureba *et al.*, 1999). However, the symptoms of PLB are generally indistinguishable from those of SLB, and the disease is considered to be a complex caused by both pathogens (Suheri and Price, 2000 a, b).

S. vesicarium, incitant of SLB of onion is considered to initiate the infection, which facilitates subsequent infection of *A. porri* causing PLB and hence, the disease is designated as purple blotch complex (Uddin *et al.*, 2006).

Similar symptoms on onion (Rao and Pavgi, 1975; Shishkoff and Lorbeer, 1989) and garlic (Rao and Pavgi, 1975; Aveling and Naude, 1992), incited by *S. vesicarium* (Basallote *et al.*, 1993), *S. botryosum* (Singh and Sharma, 1977), *Pleospora herbarum* (Conway, 1987; Falloon *et al.*, 1987) and *P. allii* (Evans and Stephens, 1984) have also been reported.

A link between *S. vesicarium* and *P. allii* was reported by Simmons (1969) on onion leaf debris.

A. porri and *S. vesicarium* often occur in the same PLB lesion and have been shown to be equally pathogenic to onions and leeks, causing a disease complex (Suheri and Price, 2000a).

Onion (*Allium cepa* cv. Giza 6) plants in several commercial fields in Egypt exhibiting symptoms of blight on the leaves and 7 seed-stalk with tip necrosis followed by small white and/or large purple spots. They have consistently isolated a fungus from the infected tissue and have identified as *S. vesicarium* based on its morphological characteristics (Hassan *et al.*, 2007).

2.4 Morphology

Ellis (1971) conducted an experiment and described that the conidia of *Stemphylium vesicarium* were oblong to ovoid, densely verrucose with 1- 5 transverse and several longitudinal septa, 25 – 40 x 13-21 µm .

2.5 Pathogenicity

Stemphylium vesicarium has been detected as both a pathogen and saprophyte on a wide range of crops. In addition to onion, *S. vesicarium* is pathogenic on garlic (Basallote, 1993), leek (Suheri and Price, 2001), Welsh onion (Misawa and Yasuoka, 2012), asparagus (Falloon, 1987), and European pear (*Pyrus communis* L.) (Llorente and Montesinos, 2006). Also, in addition to known hosts, the pathogen can cause asymptomatic infections and develop as endophytes in the living tissues of various plants (Köhl *et al.*, 2009b; Misawa and Yasuoka, 2012).

2.6 Antifungal properties of plant extract

Mulla and Su (1999); Chaturvedi *et al.* (2003), Neem (*Azadirachta indica* Juss.), the sources of biocontrol agent, has already emerged at the top of the list of plants with the highest potential as insecticides and fungicides.

Zewain *et al.* (2004); Jatav and Mathur (2005); Krishnamurthy *et al.* (2008); Wang *et al.* (2010); Obongoya *et al.* (2010); Meena (2012), The extracts from neem leaves have been used to control several fungal plant pathogens, such as *Alternaria alternata*, *A. porri*, *Aspergillus flavus*, *Fusarium oxysporum.f.sp. phaseoli*, *Fusarium solani*, *Monilinia fructicola*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*

Ergene *et al.* (2006); Mohana and Raveesha (2006), plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails .

Mathur and Sharma, (2006) the two important leaf blight diseases purple blotch and *Stemphylium* blight commonly affect onion crop in almost all onion growing pockets causing considerable damage to the crops.

Satish *et al.* (2007) tested Aqueous extract of fifty-two plant for their antifungal activity against eight important species of *Aspergillus* such as *A. candidus*, *A. columnaris*, *A. flavipes*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, and *A. tamarii* which isolated from sorghum, maize and paddy seed samples. Among fifty-two plants tested, aqueous extract of *Acacia nilotica*, *Achraszapota*, *Datura stramonium*, *Embllica officinalis*, *Eucalyptus globules*, *Lawsoniainermis*, *Mimusopselengi*, *Peltophorum pterocarpum*, *Polyalthia longifolia*, *Prosopis juliflora*, *Punica granatum* and *Syggium cumini* have recorded significant antifungal activity against one or the other

Aspergillus species tested. *A. flavus* recorded high susceptibility and hence solvent extracts viz., petroleum ether, benzene, chloroform, methanol and ethanol extracts of all the twelve plants were tested for their antifungal activity against it. Among the solvent extracts tested, methanol gave more effective than ethanol, chloroform,benzene and petroleum ether, except for *Polyalthia longifolia*, where petroleum ether extract recorded highly significant antifungal activity than other solvent extracts.

Bhosale *et al.* (2008) Botanicals, biological control agents and fungicides were tested under field conditions in India against leaf blight of onion caused by *Alternaria porri*. In botanicals, i.e. *Lantana camara* and *Pongamia pinnata* were also found effective and gave 27.08 and 22.44 per cent disease control, respectively.

Mondal *et al.* (2009) neem contains at least 35 biologically active substances of which triterpenoides, e.g. Nimbin, azadirachtin and Nimbidine are the most active fungicidal and insecticidal ingredients.

Hanaa *et al.* (2011); Gurjar *et al.* (2012); Zahid *et al.* (2012) Leaf plant extracts of *Datura stramonium*, *Eucalyptus globulus*, *Rosmarinu officinalis*, *Salix spp.*, *Ocimum spp.*, *Foeniculum* and *Cydonia oblonga* have been used successfully to control several fungal diseases in plants.

Farag *et al.* (2011) four weeks old tomato seedlings were treated with 10% of either neem and willow aqueous extracts and then infected with *Fusarium oxysporum* after 4 days of treatment. Treatments of tomato plants with neem and willow aqueous extracts reduced the percentage of disease incidence to the level of 25.5 and 27.8 per cent after 6 weeks of infection respectively. It could be reported that neem and willow aqueous extracts reduced the disease incidence of fusarium wilt in tomato seedlings by increasing the activities of antioxidant defensive enzymes and decreasing the level of lipid peroxidation.

Mishra and Gupta (2012) eight plant extracts, bio agents and fungicides were evaluated in *in vitro* conditions against purple blotch and *Stemphylium* blight of onion caused by *Alternaria porri* and *Stemphylium vesicarium*. Among the plant extracts, clove extracts of *Allium sativum* at 10 per cent resulted in maximum inhibition of growth (58.05 and 57.31 %) of *A. porri* and *S. vesicarium*, respectively followed by *Aloe vera* at 10 % (53.5 and 47.15 %).

Meena (2012) plant extracts were tested to manage purple blotch of onion caused by *Alternaria porri*, under *in vivo* and *in vitro* condition. Poisoned food technique used to screen the different extracts *in vitro* at the concentrations of 1.0, 2.0 and 5.0 percent; to measure percent inhibition of radial growth, sporulation and spore germination. It was observed that maximum inhibition (97.05 %) of mycelial growth was recorded in Palmarosa oil amended culture at 5 per cent concentration followed by *Ocimum sanctum* (95.58%) at the same concentration over check. Whereas *Eucalyptus*, *Palmarosa* and *Ocimum sanctum* oils showed minimum sporulation intensity 0.16, 0.26 and 0.39 x 10³/ml, respectively. *O. sanctum* found most effective in reducing the spore germination (75.58 %) under *in vitro* condition. In *in vivo* condition maximum reduction

(46.47 %) of disease incidence was recorded in Push sprayed plot followed by 44.75% in Perfekt and 44.23 per cent in *Polyalthia longifolia* over the check. The onion bulbs yield was also observed higher in lesser infected plots.

Ravikumar and Garampali (2013), Aqueous extracts of 39 plants selected from local flora were evaluated for antifungal potential against *Alternaria solani*, causing early blight of tomato, at 4 % concentration in Potato Dextrose Agar by poison food technique. Out of these, 13 plant extracts significantly reduced the mycelial growth of the pathogen. Inhibition rate of above 20 per cent was shown by seven plant extracts namely *Crotalaria trichotoma* (36.6 %), *Citrus aurantifolia* (27.3 %), *Azadirachta indica* (23.7 %), *Polyalthia longifolia* (23.3 %), *Datura metel* (21.3 %), *Muntingia calabura* (20.09 %) and *Oxalis latifolia* (20.09%). At 2% concentration, six extracts showed significant growth inhibition namely, *C. trichotoma* (16.6 %), *A. indica* (10 %), *Capsicum annum* (7.1 %), *D. metel* (6.6 %), *P. longifolia* (6.3 %) and *C. aurantifolia* (5.5 %).

Chetana (2013) stated that plant products and biocontrol agent has shown significant inhibition of *A.porri*. six plant products (Clerodendron, cinnamon, garlic, neem oil, pongamia oil and turmeric), five biocontrol agents (*Trichoderma harzianum*, *Chaetomium sp.*, *Beauveria bassiana*, *Verticillium lecanii* and *Metarhizium anisopliae*) was evaluated under in vitro conditions and compared with commonly used fungicides viz., mancozeb, chlorothalonil, propineb and copper oxy chloride. Among plant products evaluated, fresh aqueous extract of garlic (20 %) was effective in causing 100 per cent inhibition of mycelial growth. Neem oil and pongamia oil (20 %) caused 76.94 and 69.94 per cent inhibition. Among the biocontrol agents evaluated, maximum inhibition (79.5 %) was recorded in *T. harzianum*. Among the fungicides, mancozeb and propineb (0.3%) caused 100 per cent inhibition of mycelial growth.

Nainwal and Vishunavat (2016) studied on management of purple blotch and stemphylium blight of onion in tarai and bihar regions of Utterakhand, India. Study different bioagent and fungicides have been evaluated in field condition against both diseases. In botanicals the foliar spray of neembicide@ 0.3% disease severity of both diseases were 5.57 and 4.78 per cent, respectively

Singh *et al.* (2017) to observe the efficiency of bio-agents, botanical and fungicides against *Alternariatriticina* under in vitro and field conditions..among

botanicals maximum inhibition percent mycelia growth was observed in neem leaf extract (73.57 %) as compared to control (0). An experiment was conducted under field condition to observe the effect of bio-agents, neem leaf extract and fungicides against *Alternaria tritricina*. Eight treatments were taken up with three replications and data collected was analyzed using RBD. Maximum plant height (cm) was observed in *T. viride* (78.82 cm) followed by *T. harzianum* (78.27 cm) as compared to control (70.04 cm). *T. viride* was significantly superior as compared to other treatments.

2.8 Antifungal properties of biocontrol agents

Karthikeyan *et al.* (2006) reported that in addition to disease suppression, treatment with a mixture of antagonists promoted plant growth in terms of increased plant height and ultimately bulb yield.

Bhosale *et al.* (2008) botanicals, biological control agents and fungicides were tested under field conditions in India against leaf blight of onion caused by *Alternaria porri*. Among biocontrol, *Trichoderma viride* (38.84 %) found most effective. The sprays *Trichoderma viride* also gave significantly highest onion bulb yield of 10.31 t/ha, than the rest of the treatments.

Gupta *et al.* (2008) conducted in-vitro study for the management of soil borne fungal pathogens like *Fusarium oxysporum* and *Sclerotium cepivorum* using bio agents and oil cakes and findings indicated that *T. viride* inhibited 50.74 per cent mycelia growth as well as 93.59 per cent inhibition in sclerotia production of *S. cepivorum* while 70.40 per cent inhibition in mycelia growth of *F. oxysporum*.

Gupta *et al.* (2009) *Trichoderma viride* and *Pseudomonas fluorescens* have antagonistic capacity against plant pathogens which enhance systemic acquired resistance and plant growth promoting character. *Pseudomonas fluorescens* plays an important role in phosphate solubilization which improves the soil and plant health. Scanty information is available on quality production of onion by approaching integrated crop health and disease management

Gupta *et al.* (2011) reported that basal rot and white rot in onion can be managed by the application of *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescens*

Mishra and Gupta (2012) reported that eight plant extracts, bio agents and fungicides were evaluated in in-vitro conditions against purple blotch and stemphylium blight of onion caused by *Alternaria porri* and *Stemphylium vesicarium*. Among the bioagents, *Trichoderma viride* was effective in inhibition of growth (53.17 and 56.15%).

Prakasam and Sharma (2012) for the development of bioagents based strategy seventeen isolates of fungal and six isolates of bacterial bioagents obtained from different sources were tested for their bio efficacy against *A. porri*. Effective isolates (Th-3, Th-30, Tv-12, Tv-15, Pf-3 and Bs-2) were selected and tested for volatile and non-volatile metabolites production and vigour induction under in vitro. *Trichoderma harzianum* isolate (Th-3) expressed high level of disease reduction and growth promotion in susceptible onion (cv. Pusa Red).

Yadav *et al.* (2013) reported that the results obtained on the field efficacy of different bioagents tested, *Pseudomonas fluorescens*-I (0.5 %) was found most antifungal against *A. porri* and recorded significantly least mean disease intensity (37.19 %) and gave maximum bulb yield (27183 kg/ha).

Kamal (2014) the antagonistic activity of nine isolates of *Trichoderma* against onion purple blotch pathogen *Alternaria porri* was studied *in vitro* using dual culture assay. This study proved that *T. harzianum* as a biocontrol agent showed significant reduction in onion purple blotch disease compared with the tested fungicide.

Nainwal and Vishunavat (2016) studied on management of purple blotch and stemphylium blight of onion in tarai and bihar regions of Utterakhand, India. Study different bioagent and fungicides have been evaluated in field condition against both diseases. Among bioagents, the foliar spray of *T. harzianum* @ 1 % the disease severity for the diseases were recorded 15.44 and 13.11 per cent, respectively.

Singh (2017) to observe the efficiency of bio-agents, botanical and fungicides against *Alternaria tritricina* under *in vitro* and field conditions. Among bioagents maximum inhibition percent mycelia growth was observed in followed by *Trichoderma harzianum* (85.50 %). Among bioagents maximum inhibition percent mycelia growth was observed in followed by *Trichoderma harzianum* (85.50 %), followed by *Trichoderma viride* (83.30 %), *Pseudomonas fluorescens* (80.73 %).

2.7 Effect of biofertilizers on growth and yield of onion

Joi and Shinde (1976) reported that *Azotobacter* treatment with or without nitrogen give increased yields ranging from 18-21 percent in respect of onion crop.

Desai (1978) tested the effectiveness of *Azotobacter* strains on N-53 onion variety under field conditions and reported that of the four strains viz., A, B, C and D of *Azotobacter* tried, strain B has given maximum bulb and vegetable yield and also the size of the bulbs than other strains and control. The percentage increase in bulb yield over control ranged from 6.66 to 23.88 (strain B shown maximum i.e. 23.88 %), 11.21 to 14.01 in vegetable yield (due to strain B is 14.01 %) and 14.11 to 19.99 in bulb size (due to strain B, 19.99 %) respectively. The results in respect of bulb and vegetable yields were found to be statistically significant while that of bulb size were non-significant.

Mandhare *et al.* (1998) gave results of a greenhouse pot experiments with onion showed that application of *G. fasciculatum* + *A. chroococcum* + 50% of the recommended P rate resulted in the greatest root length, plant height, bulb girth, bulb fresh weight, root colonization and P uptake.

Sule *et al.* (2002) studied the impact of biofertilizers (*Azospirillum*, *Azotobacter*, *Rhizobium* and phosphate solubilizing bacteria) on the productivity of onion. The results are showed for onion, the average areas under biofertilizers users and non-users were 0.47 kg/ha and 0.50 kg/ha, respectively. An average productivity of biofertilizer users and non-users was 20.05 and 18.13 t/ha.

Yadav *et al.* (2003) reported that application of *Azotobacter* either in singly or in combination with 0, 50, 75 or 100 kg N per ha on the yield and yield components of onion cv. Hisar-2. Inoculation of *Azotobacter* singly or in combination with N significantly increased seed yield over the uninoculated control (by 20-31 and 32-56%, respectively). The maximum increase in plant height (10% over the control and number of umbels per plant (53% over the control) was obtained with *Azotobacter* MSX-9 in combination with 75 and 100 kg N per ha, respectively.

Aswani *et al.* (2005) noticed that effect of 4 levels of N (25, 50, 75 and 100 kg/ha) and 2 sources of biofertilizers i.e. *Azotobacter* and *Azospirillum* as seedling dipping, seed and soil treatment. The treatment combination (100 kg N + *Azotobacter* as

seedling dipping) gave the highest bulb yield and fresh weight of bulb, followed at par by (75 kg N + Azotobacter as seedling dipping).

Balemi *et al.* (2007) conducted a field experiment to test the efficacy of three Azotobacter strains as a potential supplement to nitrogenous fertilizer in improving growth and yield of onion cv. PusaMadhvi. The finding demonstrated a saving of 50 kg N ha⁻¹ without significantly affecting yield and an average increase of 13.5 per cent marketable yield due to Azotobacter inoculation in the presence of 75 kg N ha⁻¹.

Ghanti and Sharangi (2009) concluded that Azotobacter + Azospirillum combination is the best for onion as compared to others so far as the sustainability in production and environmental consideration are concerned.

Gupta *et al.* (2009) reported that the favourable nutritional environment in the root zone created by the addition of organic manures and biofertilizers resulted in increased absorption of the nutrients from soil which was responsible for increasing the yield of onion bulbs.

Waghmode *et al.* (2010) reported that Application of biofertilier (12 kg/ha Azospirillum + PSB and 20 kg /ha VAM) significantly improved growth contributing characters such as number of leaves per plant days for bulb sprouting and seed harvesting.

Yogita *et al.* (2012) did experiment on onion. The experiment comprised of four levels of chemical fertilizers and six levels of biofertilizers. The maximum plant heights, number of leaves, neck thickness, bulb diameter, bulb weight, number of scales and yield were found with the application of T₁₂ (100 kg N + 50 kg P + 70 kg K/ha + 2 kg/ha Azotobacter + 1.9 kg/ha VAM).

Yadav *et al.* (2012) concluded that application of 75 % RDF fertilizers, 25 t/ha. FYM and Azotobacter + PSB inoculation proved best in respect of B: C ratio and about 25 % nutrient required by onion crop could be supplemented by making effective combination of chemical fertilizers with FYM and biofertilizer.

Colo *et al.* (2013) Conduct an experiment to examine the effect of rhizospheric bacteria *Azotobacter chroococcum*, *Pseudomonas fluorescens* (strains 1 and 2) and *Bacillus subtilis* on the growth and yield of onion and on the microorganisms in the rhizosphere of onion and observed that the longest seedling was observed with the

application of *Azotobacter chroococcum*. The height of the plants sixty days after sowing was greater in all the inoculated variants than in the control. The highest onion yield was observed in *Bacillus subtilis* and *Azotobacter chroococcum* variants. The total number of bacteria and the number of *Azotobacter chroococcum* were larger in all the inoculated variants than in the control. The number of fungi decreased in most of the inoculated variants, whereas the number of actinomycetes decreased or remained the same.

Sharma *et al.* (2013) studied the effect of biofertilizers (*Azotobacter* and PSB) in combination with four levels of NPK fertilizers (0, 50, 75 and 100 % NPK) on growth and yield of garlic cv. „GHC-1“. They reported that the increased bulb yield was to the tune of 20.44, 15.67 and 10.43 per cent with the combination of biofertilizers (*Azotobacter* + PSB), PSB and *Azotobacter*, respectively over un-inoculated control.

Das *et al.* (2014) in their experiment on garlic included two nitrogen fixing biofertilizers (*Azospirillum lipoferum* and *Azotobacter chroococcum*) and two phosphate biofertilizers [*Vesicular Arbuscular Mycorrhiza (Glomus fasciculatum)* and phosphate solubilizing bacteria (*Bacillus polymixa*) and one potassic solubilizer (*Fraturia aurantea*) and reported that the maximum plant height (75.42 cm) and longest root (7.92 cm) were observed in NPK (100%) + *Azotobacter* + PSB + KS. The maximum leaf number (11.45), number of roots (98.46), bulb weight (29.16 g), plot yield (2.85 kg/3 m²) and projected yield (7.12 t/ha) were noticed in NPK (100%) + *Azospirillum* + PSB + KS. The next best treatment in respect of projected yield was NPK (100%) + *Azotobacter* + PSB + KS (6.64 t/ha) followed by NPK (75%) + *Azospirillum* + PSB + KS (6.28 t/ha). The results also indicated the chance of reduction of 25 % of inorganic fertilizer through inoculations of biofertilizers.

Brinjh *et al.* (2014) conducted an experiment to understand the better utilization of nutrients for growth, yield and quality improvement of onion. The result of experiment showed that maximum plant height (73.33cm) was recorded under the RDF 75% + vermicompost 25% while length of leaves (64cm), number of leaves (13.33), neck thickness (1.69cm) and number of scales were found in RDF 75% + *Azotobacter* 25%. Whereas, diameter of bulb (7.67cm), bulb length (6.80cm) and yield (42.33 t/ha) were observed in RDF 75% + phosphobacteria 25% and all the growth and yield parameters were recorded minimum under control.

3. MATERIAL AND METHODS

3.1 Material

3.1.1 Source of isolates

Infected leaves of onion (*Allium cepa* L.) were collected from the central campus, Mahatma Phule Krishi Vidyapeeth, Rahuri. From those infected samples the organism *Stemphylium vesicarium* (Wall) Simmons was isolated.

3.1.2 Culture Media

The medium used in experiment was potato dextrose agar (PDA).

3.1.3 Seeds

Disease free seeds and apparently healthy seeds of variety Phule Baswant were collected from onion breeder and seed production officer (BSP-Veg) M.P.K.V., Rahuri and used for experiment.

3.1.4 Culture of bio-control agent

Pure culture of *Trichoderma viride*, *T. harzianum*, *T. hamatum*, *T. konnigiia* and *Pseudomonas fluorescense* were obtained from the Department of Plant Pathology and Agricultural microbiology, M.P.K.V., Rahuri and taken for experimental studies.

3.1.5 Glasswares

Petriplates, test tubes, conical flasks, glass slides, cover slip, glass rods, beakers etc. were used in the experimental work.

3.1.6 Equipments

Laboratory equipments used in the experiment were autoclave, hot air oven, research microscope, incubator, laminar air flow, refrigerator, isolation chamber, electrical balance etc.

3.1.7 Biocontrol agents

Table 1. Biocontrol agents used for the *in vitro* bioassay

Sr. No.	Biocontrol agents	Concentration (%)
1	<i>Trichoderma viride</i>	0.5
2	<i>Trichoderma harzianum</i>	0.5
3	<i>Trichoderma hamatum</i>	0.5
4	<i>Trichoderma konnigii</i>	0.5
5	<i>Pseudomonas fluorescense</i>	0.5

Table 2. Medicinal plant and plant parts used for experiment

Sr. No.	Botanical name	Common name	Parts used
1	<i>Azadirchata indica</i>	Neem	Leaves
2	<i>Latena camera</i>	Ghaneri	Leaves
3	<i>Ocimum sanctum</i>	Tulas	Leaves
4	<i>Pongamia pinnata</i>	Karanj	Leaves
5	<i>Aloe vera</i>	Korphad	Leaves
6	<i>Ginger officinalis</i>	Ginger	Rhizome
7	<i>Allium cepa</i>	Onion	Bulb
8	<i>Allium sativum</i>	Garlic	Clove
9	<i>Datura stromium</i>	Datura	Leaves
10	<i>Parthenium hysterophorus</i>	Gajar gawat	Leaves

3.1.9 Miscellaneous material

The miscellaneous materials viz. earthen pot, plastic petri dishes, blotting paper, needle, spirit lamp, mortar and pestle, filter paper, sterilized water, cork borer, muslin cloth, cotton wool, alcohol etc. were used during experimentation.

3.2 Method

3.2.1 Isolation and identification of causal agent

The fresh diseased leaves showing typical *Stemphylium* blight symptoms were collected from onion field and brought to plant pathology laboratory. Slides were prepared by mounting small pieces of diseased leaf tissue in a drop of water and observed microscope for presence fungal spore and mycelium. The diseased leaves were then washed under tap water and air dried. The infected leaves were then cut into small pieces in a manner that each piece covered half healthy and half diseased leaf portion.

The piece were then surface sterilized in $HgCl_2$ solution for one minute, washed with three changes of sterilized water to remove traces of mercuric chloride and were dried on sterilized blotting paper. Then the leaf pieces were aseptically placed on PDA medium already poured in sterilized petridishes. The plates were

incubated at temperature of $28\pm 1^{\circ}\text{C}$ and were observed periodically for fungal growth, till visible colony appeared around the leaf pieces.

This growth was then aseptically transformed on PDA slants with the help of sterilizing inoculating needle. This culture was then maintained as stock for further studies. Identification of fungal isolates of pathogen was carried out based on macro and microscopic characterization (Ellis, 1971). The pure identified culture of the fungus was transferred to PDA slants and kept in refrigerator at 4°C for further studies.

3.2.2 Maintenance of pure culture

Isolated and purified pathogen was sub cultured on PDA slants and kept at $28\pm 1^{\circ}\text{C}$ for seven to eight days for good growth. Such slants were preserved in refrigerator at 5°C to 10°C and the isolates was sub cultured once in month and used for further studies. Purified culture was obtained by single spore isolation.

3.2.3 Pathogenicity

3.2.3.1 Inoculation

Symptomatology of the disease was studied under glass house condition by following leaf inoculation technique. Pathogenicity of the fungus was studied by raising one and half month onion seedling in a pot under greenhouse condition. After one month of transplanting conidial suspension of *Stemphylium vesicarium* grown on potato dextrose broth in conical flask, stained through muslin cloth and sprayed on plants by using hand atomizer. Before spraying the conidial suspension leaves were injured slightly by making hole with carborandum powder and sticker (sandovit 0.2%) was added to conidial suspension 24 hours before and after spraying of inoculum potted plants was observed for development of disease regularly.

3.2.3.2 Development of symptoms

Pots were regularly watered and observed daily for the developments of symptoms. Symptoms of Stemphylium blight due to *Stemphylium vesicarium* was recorded and given under results.

3.2.3.3 Reisolation

Reisolation were made from artificially inoculated plants, showing symptoms of infection, by using tissue isolation method. Culture obtained from reisolation transferred on PDA slants for its conformation with original isolates, to prove the Koch's postulates.

3.2.4 Methods of plant extraction

Ten plants were selected (Table-2) to estimate the antifungal behavior against *Stemphylium vesicarium* of onion through poison food technique (Nene and Thapliya, 1976). The plant species and parts used with their concentration are shown in Table 2. The fresh leaves and other parts of healthy plants were collected and thoroughly wash with tap water and air dried. 10 gm of plant material was weighed, the plant material was then crushed in mortar and pistle by adding 10 ml of sterile water (1:1 w/v). The extract was filtered through muslin cloth. The supernant was taken as standard plant extract solution (100%). Further extract was diluted by adding sterilized water to get 10 per cent concentration.

3.2.5 Preliminary screening of plant extract for their antifungal properties against *S. vesicarium*

Plant extract by crude extraction, where screened against test fungus as follows. Besides plant extracts at 10 per cent were also tested *in vitro* by poisoned food technique. The plant extracts were subjected to boiling temperature of 50°C in water bath to avoid contamination and then they incorporated into PDA media by transferring 2 ml of each type of plant extract into a petri dish containing 20 ml melted warm PDA medium and they were gently shaken for through mixing of the extract. The PDA plates containing the plant extract were inoculated aseptically with *S. vesicarium* by transferring 0.5 cm diameter agar disc of 7 to 10 days old culture of the pathogen to the center of petri dish. Three replication were maintained for each treatment. The basal medium PDA without any phytoextract served as control. All the inoculated petri dishes were incubated at 28±1°C. The radial growth of the test fungus in the trated plates was measured in all treatments when the pathogen growth touched the edges of petri dishes.

The per cent inhibition of fungal growth was estimated by using the formula given by Vincent (1927).

$$I = \frac{C - T}{C} \times 100$$

Where, I- per cent inhibition, C- colony diameter in control, T- colony diameter in treatment

3.2.6 Evaluation of different antagonists against *S.vesicarium in vitro*.

The fungal bio-control agent, namely *Trichoderma viride*, *T. harzianum*, *T.hamatum*, *T.konnigii*, and *P.flourescence* were obtained from the Department of Plant Pathology and Agricultural Microbiology, M.P.K.V.,Rahuri. These bio-control agent were screened under *in vitro* condition against *S. vesicarium* for their antagonistic activity by using dual culture technique. To study the antagonistic effect, an experiment was carried in petriplates poured with sterilized PDA. These fungal bio agent were grown on seperat PDA plates for 7 days. The disc of 5 mm diameter were cut with which where already poured with PDA. The disc of the test fungus was placed exactly opposite to the disc of bio agents. They were placed in such a manner that both the test fungus and bio agent would get an equal appportunity for their growth and incubated at temp 28±1°C. The experiment was conducted with three replication similarly, bacteria were streaked on the opposite site of the pathogen. Control plate contained only the test fungus without bio agent. The observation were recorded when the mycelial growth in the control plates reached the edges of petri plates.

Per cent inhibition of growth was calculated by following formula given by Vincent (1927).

$$I = \frac{C - T}{C} \times 100$$

Where, I- per cent inhibition, C- colony diameter in control, T- colony diameter in treatment

3.2.7 Field experiment

3.2.7.1 Site

The experiment was conducted on field of plant pathology and agril. Microbiology, M.P.K.V., Rahuri.

3.2.7.2 Layout of experiment

A field experiment to test efficiency of botanicals and bio-control agents against stemphylium blight of onion was conducted during kharif 2017.

1. Design : Randomized Block Design
2. Replication : Three
3. Area under experiment : 273 m²
4. Plot size : 3.00 X3.00 m²
5. Variety : Phule baswant
6. Date of transplanting : 16. 06. 2017
7. Treatment : 7

(2 plant extracts, 2 bio-control agents, and 2 bio fertilizers and 1 control)

Treatment Details

Treatment No.	Name of treatment	Concentration
T ₁	<i>Allium sativum</i>	10 %
T ₂	<i>Aloe vera</i>	10 %
T ₃	<i>T.viride</i>	0.5 %
T ₄	<i>T.harzianum</i>	0.5 %
T ₅	Azotobacter	25 g/kg
T ₆	PSB	25 g/kg
T ₇	Control	

Observation recorded as

- i. First observation : 16.07.2017
- ii. Second observation : 01.08.2017
- iii. Third observation : 16.08.2

iv. Forth observation : 01.09.2017

Interval between each observation was 15 days

R1			R2			R3		
T7			T5			T1		
T3			T1			T4		
T5			T6			T7		
T1			T3			T2		
T4			T7			T5		
T6			T2			T3		
T2			T4			T6		

3.2.7.3 Seed treatment with biofertilizer

In T₅ seeds are treated with azatobactor @ 25 g/kg of seed. T₆ seeds are treated with PSB @ 25 g/kg of seeds.

3.2.7.4 Spraying of botanicals and bio-control agent

The experimental plots in field trials were visited daily to observe incidence of disease. The disease incidence was noticed on 45th day after spraying were

carried out with bio agent under test at 50th, 65th and 80th days after transplanting. A sticker sandovit (0.1%) was added to avoid immediate runoff of spray solution as the leaf surface of onion is glossy.

3.2.7.5 Observation

The observations were recorded for all three replications and the average was calculated. Per cent disease intensity was recorded eight after third spraying.

The per cent disease intensity was recorded on the basis of leaf area infected, taking into consideration whole leaf area as 100 per cent. The disease intensity for stemphylium blight is rated using 0-5 scale (Sharma, 1986).

Rating number Reaction description

- | | |
|---|---|
| 0 | No disease symptoms |
| 1 | A few spots towards tip covering 10% leaf area |
| 2 | Several purplish brown patch covering upto 20 % of leaf area |
| 3 | Several patches with paler outer zone covering area upto 40 |
| 4 | Leaf streaks covering upto 75% leaf area or breaking of leave from the center |
| 5 | Complete drying of the leaves or breaking of leaves from center |

Per cent Disease index (PDI) was calculated by using formula given by Wheeler (1969). The data were statistically analyzed.

$$\text{PDI} = \frac{\text{Total sum of numerical rating}}{\text{Number of observation}} \times \frac{100}{\text{Maximum disease rating}}$$

$$\text{PDC} = \frac{\text{PDI in check} - \text{PDI in treatment}}{\text{PDI check plot}} \times 100$$

3.2.9 Statistical analysis

All the per centage was converted into arcsin values. The final data of per cent disease intensity, per cent disease control, per cent increase in yield, no. of leaves, weight of bulb height of plants and yield of onion bulbs was subjected to statistical analysis.

The standard error (S.E.) for disease intensity, disease control and yield of onion bulbs was calculated. For comparing two treatments a mean critical difference (C.D.) at 5 per cent of significance were worked out.

4. RESULTS AND DISCUSSION

4.1 Isolation, pathogenicity, and identification

4.1.1 Microscopic examination

The microscopic examination of slides prepared from the leaf tissue showing typical *Stemphylium blight* symptoms revealed the presence of spores of *Stemphylium vesicarium*.

4.1.2 Isolation of organism

The organism isolated from diseased onion leaves showing typical *Stemphylium* blight symptoms on PDA medium.

4.1.3 Establishment of pure culture

The pure culture of the organism was established by single spore isolation technique and the culture was maintained on PDA slants, used for further studies.

4.1.4 Identification of causal organism

Organism isolated and established in pure culture was identified as *Stemphylium vesicarium* (Wallr) Simmon, causing *Stemphylium* blight in onion. The mycelium was observed as filamentous, septate and light brown in colour. Conidiospores were straight to variously curved, simple or occasionally a one branch, cylindrical but enlarging apically to the site of conidial production, whereas, conidiospores were dilute yellow brown or olive brown, smooth 1-4 septate 5-8 x 33-47 µm with swollen apical cells or distingly flared to 7-9 µm in diameter, apical sporiferous cells with a single spore 4-7 µm in diameter having as many as five apical proliferation.

4.2 Pathogenicity

4.2.1 Inoculation

To prove the pathogenicity, leaf inoculation technique was used. Injured and artificially inoculated onion plants showing initial symptoms appear on the leaves brown spots, often surrounded by a halo on leaves measuring 1-2mm in diameter on inoculated leaves within 4 to 5 days. Within one month after inoculation, these spots coalensced to form large brown and finally black as the fungus sporulates. These symptoms were also observed on uninjured and artificially inoculated onion leaves.

However, the development of initial symptoms was delayed by 4 to 5 days in case of uninjured leaves as compared to injured and inoculated leaves. Further development of larger patches on uninjured and inoculated plants was observed 40-45 days after inoculation. This indicated the injury caused rapid development of symptoms. The symptoms observed on artificially inoculated plants were relatively same with those observed under the natural infection in the field. Uninoculated (injured and uninjured) remained healthy. The conidia produced on artificially inoculated onion leaves were observed under microscope by mounting on slides. They were same in morphology to those observed on naturally infected leaves.

S.vesicarium is pathogenic on garlic (Basallote 1993), leek (Suheri and Price 2001), Welsh onion (Misawa and Yasuoka 2012), asparagus (Falloon 1987), and European pear (*Pyrus communis* L.) (Llorente and Montesinos 2006). Also, in addition to known hosts, the pathogen can cause asymptomatic infections and develop as endophytes in the living tissues of various plants (Köhl *et al.* 2009b; Misawa and Yasuoka 2012).

4.2.2 Reisolation

The symptoms observed during pathogenicity test were identical to those recorded on the field sample collected.

4.3 Inhibition of mycelial growth

The effect of antifungal property from crude extracts of ten medicinal plants, five bio-control agents against *Stemphylium vesicarium* was studied by poisoned food techniques and dual culture technique on solid PDA media. At the same control set was also maintained for comparison.

4.3.1 In vitro studies

The results regarding mycelial growth in different crude extracts of botanicals and bio-control agent are given in table 3 and graphically represented in fig 1. From the data it was seen that all the treatments were significantly effective over control treatment in reducing the mycelial growth of the pathogen. Antifungal activity of ten botanicals extracts was assayed and data of plant extracts on the growth of *S. vesicarium* is presented in table 3.

Per cent inhibition of *Stemphylium vesicarium* by different botanicals was recorded along with mean colony diameter. Maximum diameter of colony (90 mm) was

recorded in control followed by *Aloe vera* (47.13 mm). In botanicals minimum pathogen growth (12.62 mm) was recorded in *Allium sativum* followed by *Aloe vera* (16.35 mm) were observed to be at par in effectiveness in controlling the vegetative growth of pathogen. Statistical analysis of per cent inhibition revealed that treatment of *Allium sativum* (85.97 %) which was followed by *Aloe vera* (81.85 %) (Table 3). Minimum pathogen growth (10.52 mm) was recorded with *Trichoderma viride* followed by *T. harzianum* (11 mm). Statistical analysis of per cent inhibition revealed that treatment of *Trichoderma viride* (T₂) was found most effective by giving 88.31 per cent of inhibition of pathogen, followed by T₁ (87.77 %) (Table 4).

Table 3. *In vitro* effect of different bio agents against leaf blight of onion

Tr. No.	Treatment	Mean colony diameter (mm)	Per cent inhibition (%)
T ₁	<i>Azadirchta indica</i>	38.72	56.97
T ₂	<i>Lantana camera</i>	17.41	80.65
T ₃	<i>Ocimum sanctum</i>	39.75	55.83
T ₄	<i>Pongamia piñata</i>	32.93	63.30
T ₅	<i>Aloe vera</i>	16.35	81.85
T ₆	<i>Ginger officinalis</i>	31.41	65.10
T ₇	<i>Allium cepa</i>	47.13	47.63
T ₈	<i>Allium sativum</i>	12.62	85.97
T ₉	<i>Datura stramonium</i>	30.75	65.83
T ₁₀	<i>Parthenium hysterophorus</i>	18.72	79.20
T ₁₁	Control	90	
	S.E.	1.74	
	C.D. at 5%	5.10	
	C.V.	8.82	

The similar effect were also observed in the present studies in tune with the finding of various workers Mohamood *et al.* (1995), Zewain *et al.* (2004), Jatav and Mathur (2005), Ergene *et al.* (2006), Mishra and Gupta (2008), Bhosale, *et al.* (2008), Mondal *et al.* (2009), Wagh *et al.* (2010), Hanaa *et al.* (2011), Guljar *et al.* (2012),

Prakashan and Sharma (2012), Mishra and Gupta (2012) and Zahid *et al.* (2012). Antifungal activity of ten botanical aqueous extracts were evaluated under *in vitro* condition. The significant reduction in growth of *S. vasicarium* were observed in respect of all the plant extracts tested. Results indicated that the clove extracts of *Allium sativum* at 10% concentration caused maximum inhibition (85.97 %) which was followed by *Aloe vera* (81.85 %).were also found effective in reducing the growth of the pathogen under *in vitro* condition. Similar results were obtained by Prasad and Barvwal (2004), Gupta *et al.* (2008), Gupta *et al.* (2009), Prakasham and Sharma (2012), Mishra and Gupta (2012), Yadav *et al.* (2013) and also reported in other host by Bhosale *et al.* (2008), Meena (2003).

Table 4. *In vitro* effect of different bio-control agents against leaf blight of onion. Per cent inhibition

Tr. No.	Treatment	Mean colony diameter (mm)	Per cent inhibition (%)
T ₁	<i>Trichoderma harzianum</i>	11.00	87.77
T ₂	<i>Trichoderma viride</i>	10.52	88.31
T ₃	<i>Trichoderma hamatum</i>	35.73	60.30
T ₄	<i>Trichoderma konnigii</i>	35.46	60.60
T ₅	<i>Pseudomonas flourosence</i>	23.76	73.60
T ₆	Control	90.00	
	S.E.	2.12	
	C.D. at 5%	6.55	
	C.V.	10.70	

4.4 Evaluation of botanicals and bio-control agents against *Stemphylium* blight of onion in field

The results of spray treatments of plant extracts of botanicals and bio-control agent against *Stemphylium* blight of onion under field condition after each spray condition was given in Table . The disease was observed on 45 days after transplanting.

4.4.1 Per cent disease intensity after first spraying

All botanicals and bio-control agents are found significantly reduced disease intensity as compared to control treatment. A bio-control agent *Trichoderma*

viride was significantly superior to control and other treatment. The lowest disease intensity (9.80%) was recorded in *Trichoderma viride* against (27.30%) in control treatment. This was followed by *T.harzianum*, which was recorded 15.50 per cent disease intensity and was at par with botanical plant extracts of *Allium sativum* (19.50%) and *Aloe vera* (20.40%) and were significantly superior over the rest of treatment. The highest per cent of disease intensity (27.30%) was recorded in unsprayed control treatment. The condition after first spray was given in Table 5.

Table 5. Efficiency of botanicals and biocontrol agents against *Stemphylium* blight of onion per cent disease intensity and per cent disease control. (After first spraying)

Tr. No.	Treatment	First observation		Second observation		Third observation	
		PDI	PDC	PDI	PDC	PDI	PDC
T ₁	<i>Allium sativum</i>	19.50	36.63	28.80	27.63	37.62	27.38
T ₂	<i>Aloe vera</i>	20.40	55.27	29.30	46.70	39.35	25.63
T ₃	<i>Trichoderma harzianum</i>	15.50	43.22	22.40	43.71	31.52	38.41
T ₄	<i>Trichoderma viride</i>	9.80	64.10	18.20	54.27	25.35	51.27
T ₅	Azotobactor	21.70	24.17	31.26	23.35	45.21	16.90
T ₆	PSB	20.80	20.14	33.65	15.45	41.49	19.30
T ₇	Control	27.30		39.80		51.35	
	S.E. $\pm$					1.04	
	CD at 5 %					3.22	
	CV (%)					6.13	

4.4.2 Per cent disease intensity after second spraying

The condition after second spray was given in Table 5. The lowest per cent disease intensity (18.20%) was recorded in the treatment of *Trichoderma viride* and was at par with *Trichoderma harzianum* (22.40%).

These treatments were significantly superior over rest of the treatments in recording in recording the per cent disease intensity. The highest per cent disease intensity (39.80%) was recorded in control treatment. The per cent disease intensity in remaining plant extracts as per descending order were, *Allium sativum* (28.80%), *aloe*

vera (29.30%). The per cent disease intensity in biofertilizer, azatobactor (31.26%), PSB (33.65%).

4.4.3 Per cent disease intensity after third spraying

From the data presented in Table 5, it was seen that the spray treatment of *T. viride* was most effective and significantly superior among all the treatments which recorded the lowest per cent disease intensity of 25.35 per cent and gave 51.27 per cent disease control. The biocontrol agent *T. harzianum* was the second best treatment which recorded 31.52 per cent and gave 38.41 per cent disease control. Among the botanicals minimum per cent disease intensity 37.62 per cent was recorded in *Allium sativum* and maximum disease intensity was (51.35%) was recorded in control treatment.

The similar results reported in other host by Bhosale *et al.* (2008), botanicals, bioagents and fungicide were tested under field condition. *Trichoderma viride* most effective. Also reported by Mohan *et al.* (2001), Kartikeyan *et al.* (2006), Meena (2012), Mishra and Gupta (2012) Yadav *et al.* (2013), Deepa Nainwal and Karuna Vishunavant (2016), Sardar Singh Kakraliya *et al.* (2017).

The biofertilizer, Azatobactor and PSB also effective compared to control treatment, which give 16.90 per cent and 19.30 per cent disease control respectively. Joi and Shinde (1976), Desai (1978), Sule *et al.* (2002), Yadav *et al.* (2003), Balemi *et al.* (2007), Cole *et al.* (2013), Yadav *et al.* (2012), Sharma *et al.* (2013) and Brinjh *et al.* (2014).

4.5 Efficiency of botanicals and bio control agents on yield of onion bulb

From the results, presented in Table 6, it was observed that the difference between the yield of onion bulb by effects of botanicals and biocontrol agents were significant.

The highest yield (10.57 t/ha) was obtained from treatment *Trichoderma viride* followed by *T. harzianum* which recorded 10.44 t/ha. All treatment yielded more or less higher yield ranging from 8.20 to 10.57 t/ha than control treatment. The lowest bulb yield of 8.04 t/ha was recorded in untreated control treatment.

Table 6. Effect of botanicals and biocontrol agents on yield of onion crop (t/ha)

Tr. No.	Treatment	Yield (t/ha)
T ₁	<i>Allium sativum</i>	8.71
T ₂	<i>Aloe vera</i>	8.53
T ₃	<i>Trichoderma harzianum</i>	10.25
T ₄	<i>Trichoderma viride</i>	10.57
T ₅	Azatobactor	8.21
T ₆	PSB	8.33
T ₇	Control	8.04
	General mean	8.95
	S.E. $\pm$	0.13
	CD at 5 %	0.40
	CV (%)	2.54

5. SUMMARY AND CONCLUSION

Stemphylium blight is a severe disease of onion in Maharashtra. The causal organism *Stemphylium vesicarium* (Wallr). Simmons was isolated from infected plant leaves collected from field. The isolated fungus grew in the form of colonies were olive brown to black, somewhat velvety on PDA. Mycelium of the fungus was branched, raised, oblong to ovoid, dark green to black in colour with faint yellow pigmentation in the center of the medium with 1-5 transverse and several longitudinal septa, 25- 40 X 13- 21µm. (Ellis 1971).

Blight symptoms were observed from 30 to 35 days of growth, but it was more prominent at 50 days onwards. The infected plants initially exhibited characteristic symptoms of tip necrosis with white flecks that enlarged and produced sunken purple lesions, which were surrounded by a yellow to pale brown border. Later, small light irregular to oval, yellow to brown, water soaked and non-delineated lesions developed on older leaves. Lesions usually turn light brown to tan at the centre and later dark olive brown to black. In view of the commercial importance of the crop and losses caused by disease in the field, the present study was undertaken.

The objective of the study were; to study the isolation; identification and pathogenicity of causal organism; to see the effect of plant extract, bio control agents and bio fertilizer against *Stemphylium* blight of onion under *in vitro* and under field condition. The pathogenicity of the organism isolated was confirmed by undertaking the experiments on healthy onion plants of which the Koch's postulate were fully satisfied. From this it was concluded that fungus *Stemphylium vesicarium* is the potential pathogen of *Stemphylium* blight disease of onion.

In the present study, the aqueous plant extract of ten medicinal plant., viz., *Azadirchta indica*, *Lantana camera*, *Ocimum sanctum*, *Pongamia piñata*, *Aloe vera*, *Ginger officinalis*, *Allium cepa*, *Allium sativum*, *Datura stramonium*, *Parthenium hysterophorus*, and five bio control agents viz *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma hamatum*, *Trichoderma konnigii* and *Pseudomonas* fluorescence were tested against *Stemphylium vesicarium* for, their antifungal activity in *in vitro* condition. Among these the inhibition of mycelial growth by aqueous extracts of all ten

plants and bio control agents were studied by employing poisoned food techniques on PDA media. The *Trichoderma viride* was found significantly superior in inhibiting the growth of *S. vesicarium* followed by *Trichoderma harzianum* over control. Among plant extracts *Allium sativum* and *Aloe vera* inhibited mycelial growth of fungus very effectively.

Further extract of these plants and bio control agents, which are significantly superior and bio fertilizer were also studied under field condition. In field also with three sprays at 15 days interval it was observed that *T. viride* and *T.harzianum* were superior to control. Among plant extracts *Allium sativum* and *Aloe vera* gave most effective results. Also in bio fertilizer, *Azotobactor* and PSB gave effective results over control in per cent disease intensity and per cent disease control.

CONCLUSION

Among biocontrol agent *T. viride* and *T.harzianum* at 0.5 % showed maximum growth of inhibition. Garlic clove extracts at 10 % showed maximum growth inhibition among the plant extracts. Thus, the study indicated that suitable intergration of more efficient ecofriendly treatments like bio agents and botanicals, bio fertilizer may provide a better management of the *Stemphylium* blight of onion

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7. APPENDICES

Potato dextrose agar

Sr.no.	Material	Quantity
1.	Pealed potato	250 gm
2.	Dextrose (C ₆ H ₁₂ O ₆)	20 gm
3.	Distilled water	1000 ml

8. VITAE

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MASTER OF SCIENCE (AGRICULTURE)
 in
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