

Original Research Article

Influence of Bio-pesticides and Fungicides on Disease Development of Garlic under *invitro* and *invivo* Conditions

Prahlad, Anupriya*, S. L. Godara and Usha Bhale

Department of Plant Pathology, College of Agriculture, Jawahar Lal Nehru Krishi
Viswavidhalya, Jabalpur, M.P., India

*Corresponding author

ABSTRACT

A study was conducted in the Department of Plant Pathology, College of Agriculture, Jawahar Lal Nehru Krishi Viswa vidhalya, Jabalpur (M.P.) during Rabi season 2015-2016. Among the three bio-pesticides (*Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescenc*) and four fungicides (Carboxin + Thirum, Carbendazim + Mancozeb, Copper oxy chlorid, Copper hydroxide) tested was *in-vitro*, *in-vivo* and mycoflora conditions. Management approaches for purple blotch, stemphyllium blight, basal rot, rust, garlic mosaic virus, downy mildew have been worked out in various agro ecological zones of India. However *in- vitro* revealed that in maximum emergence was observed in clove, treated with Carboxin+Thirum (as Vitavax power 200, commercial formulation)@ of 3.0g /kg clove, and minimum observed by *Pseudomonas fluorescenc*. A field experiment was conducted whereas maximum emergence 89% was recorded in clove treated with Carboxin + Thirum and Carbendazium+ Mancozeb @ of 3.0 g/kg clove. Reduced incidence of all diseases were noticed due to clove treatment with fungal bio-pesticides and fungicides as compared to bacterial bio-pesticides.

Keywords

Bio-pesticide,
Fungicide, Pink
rot, White rot,
Purple blotch,
Stemphyllium
tender tip blight

Introduction

The garlic (*Allium sativum* L.) is useful to mitigate the effect of medicinal and culinary properties throughout the world. Biochemical constituents including thiosulfates, thiosulfonates, allicin, ajonen that make the crop very precious in human health care system making it exclusive medicinal commodity. Effective antimicrobial properties of garlic have been well accepted. The crop has exhibited a potential therapeutic medicinal value with antifungal, antibacterial, antiviral, anti helmantic, antiseptic and anti-inflammatory properties. The most recent classification scheme of garlic is class

Liliopsida, subclass Liliidae, superorder Liliianae, order Amaryllidales, family Amaryllidaceae subfamily Allioideae, tribe Allieae and genus *Allium* which is mainly based on the sequences of nuclear ribosomal DNA (Reuter *et al.*, 1996).

Garlic contains at least 33 sulfur compounds, several enzymes and the minerals germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc; vitamins A, B1 and C, fiber and water. It also contains amino acids lysine, histidine, arginine, aspartic acid threonine, glutamine, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tryptophan and

phenylalanine garlic extract is effective against bacteria, fungi, parasites, lower blood pressure, blood cholesterol and blood sugar, prevent blood clotting, protect the liver and contains antitumor properties in humans garlic extracts have exhibited activity against Gram Positive and Gram Negative bacteria. It is used as fresh juice, aqueous and alcoholic extracts, lyophilized powders, steam distilled oil and several other commercial preparations for the wellness, curative and preventive medicines in humans (Sovova and Sova, 2004; Fenwick and Hanely, 1985; Derese, 2010).

Garlic is a herbaceous annual bulbous plant in the family Amaryllidaceae grown for its pungent, edible bulb. The garlic plant can either have a short, woody central stem (hard neck) or a softer pseudostem made of overlapping leaf sheaths (soft neck). India rank second with 2.0 lakh ha area and second in production with 10.58 lakh ton at global level, however, remains very low in productivity with 5.05 t/ha as compared with Egypt (25.28 t/ha) and China (23.60 t/ha). Long day and Short day type of garlic are cultivated in India. In Madhya Pradesh short day type varieties are grown. Long day type of garlic is confined to hills of India, especially in Jammu and Kashmir, Himachal Pradesh and Uttarakhand region. During 2014-2015, garlic was grown on 2.62 lakh ha with a total production of 14.25 lakh tons Madhya Pradesh, Gujarat, Rajasthan, Uttar Pradesh, Assam, Punjab, Maharashtra, (Source: NHRDF, Nashik, 2015). In Madhya Pradesh area and production was (81.17 lakh ha with total production 4.26 lakh tons during 2014-2015).

Worldwide, garlic was grown over 14.22 lakh hectares and had a total production of 237.70 lakh tons and an average productivity of 16.71 t/ha (Source: FAOSTAT, 2013). Among 140 countries where garlic is grown,

China is world leader in production (80.92%), followed by India (4.45%). Per hectare productivity of garlic is the highest in Egypt (24.36 t/ha).

Investigation on various aspects of Purple blotch (*Alternaria porri*) (Dhiman *et al.*, 1986; Quadri *et al.*, 1982); Stemphylium blight (Thind *et al.*, 1985; Singh *et al.*, 1977), basal rot (*Fusarium oxysporum*) (Mathur and Sankhala, 1963) Rust (*Puccinia porri*) (Sandhu and Kang, 1988); garlic mosaic virus (Ahlawat, 1974), downy mildew (Singh *et al.*, 1987) are reported from India. Management approaches for purple blotch; Stemphylium blight, basal rot, rusts; garlic mosaic virus, downy mildew has been worked out in various agro ecological zones of India (Shrivastava *et al.*, 1992). The garlic crop is cultivated in several countries and susceptible to number of diseases at various stages of plant growth (Walker, 1952). From different parts of the world, downy mildew, rust, purple blotch; Stemphylium blight, basal rot, have been observed leading to substantial losses (Ahmad and Karimullah, 1998; Apaza and Matos, 2000; Schwartz and Mohan, 1995; Evarts and Lacy, 1990).

Various diseases have been reported on garlic bulbs in particular are affected by association of number of fungal pathogens both in fields and storages. The bulbs due to handling, cultivation practices and ill storage are infected severely by number of fungal pathogens. The bulbs are significantly damaged and destroyed resulting in bulb rot and bulb necrosis (Rai and Agarwal, 1976).

Symptoms

The disease may appear at any stage of growth, provided an environmental condition is favourable. Infection of seedlings occasionally occurs; however, the first infections are normally detected in plants

bearing three to five leaves. Initial stages of infection are confined to the root system and base plate. The first above-ground symptoms of infection include a yellowing of leaves beginning at the tips and progressing downward. A gradual decline in the plant continues for some days or weeks and in the case of young plants may constitute a rapid wilt and collapse of aerial parts ultimately the entire plant is killed. On underground parts the fungus itself is visible as superficial, fluffy white mycelium. The roots are gradually destroyed and the fungus causes a soft, watery decay of the bulb commencing at the base plate (Scott, 1956; Walker, 1924).

Mustard seed sized sclerotia, normally 200-500 µm diameters, are formed on the clove base and within decaying root and stem tissue. Above-ground symptoms are not normally evident until the pathogen has colonized and partially rotted the stem and leaf sheaths. Roots frequently extend horizontally, providing a direct path for mycelial growth to nearby plants. Infected plants therefore tend to occur in clusters from a few up to 40 or more adjacent plants (Metcalf *et al.*, 1997; Crowe and Hall, 1980).

Identification of diseases at field level

White rot

The initial symptoms of white rot disease were the yellowing of leaves and later roots were destroyed. The leaves of infected plant exhibited girdling and dieback. Leaf decay at the base was observed and older leaves collapsed first. A semi watery decay of the stalks of bulb was recorded. The infected plant was easily pulled out from ground. Root was rotted. White fluffy growth around the base of the bulb was observed. The white fluffy fungal growth became more compact as the disease progressed, at later stage numerous small spherical black bodies

(Sclerotia) formed on the mycelial mat, the sclerotia were approximately of the size of pin head, poppy seed, resembling mustard grain, the bulbs became soft and water soaked. Based upon the fungal mycelium and sclerotium the fungus was identified as *Sclerotium ceviporum*.

Pink rot

The garlic affected by basal rot pathogen exhibited progressive yellowing. Affected roots were brown to dark pink. In severe condition of infection white fungal growth was noticed at the base infected bulb, when the infected bulb cut vertically a brown discoloration on the spilt was apparent. In some cases stem plate tissue became pitted and showed dry rot. Under dry conditions the stem plate lead to crick scales. In advanced stage the bulb started decaing from lower and ultimately whole plant died. On the basis of fungal characteristics the pathogen was identified as *Fusarium oxysporum*.

Purple blotch

The purple blotch symptoms were noticed on stalks as small sunken whitish flacks, with purple colour. Later the lesion girdled leaves and stalks leading the drooping. Oval shaped tan and deep purple lesions on leaves margin were recorded. Concentric zone were observed within the lesions. The initial symptoms of purple blotch were small water soaked lesion that appeared on older leaves. As the disease progressed the lesion on larged became yellow and concentric ring formed on margins. Based on the fungal characteristics the pathogen was identified as *Alternaria porri*.

Stemphylium tender tip blight

The symptoms appeared as small yellow to orange flacks which turned brown, extended

along the blade in both direction from the lesions. In advanced stages lesions girdled and killed leaves and stem, due to infection of *Stemphylium vesicarium*, under field conditions. Purple blotch and Stemphylium disease were differentiated on basis of margins of the lesions.

In Stemphylium lesions were elongated spherical shaped surrounded in pinkish margin while in purple blotch small sunken whitish flacks with purple colour centres and the lesions were surrounded by yellow hallow.

Identification of pathogens

Purple blotch (*Alternaria porri* Ellis cif.)

The conidiophores of the fungus arose single or in groups, straight, often, geniculate, septate, pale to mild brown in colour, measuring upto 120 µm length and 5-10 µm width.

Conidia were muriform and usually solitary, straight, curved, obclavate and taper to peak that was commonly about same length, and are slightly larger than conidia, the conidia were brown in colour, smooth and measured 100 to 300 µm in length with the broadest part 15 to 20 µm thick. Each conidium had 8 to 12 transverse and upto 7 several longitudinal or oblique septa.

Tender tip blight (*Stemphylium vesicarium* (Wallr) E Simmons (Tel. *Pleospora alli* (rabenh) Ces and de acot)

The conidia of the fungus were oblong, broadly, oval measuring 12 to 22 into 25 to 42 µm in size, with 1-6 transverse septa, and 1-3 longitudinal septa. Conidia were constricted at the transverse septa. Conidia were light to medium golden brown to olive brown, each had a conspicuous basal scar

like zone. Conidiophores were straight to curved, 1-4 septate measuring 5-8 into 33-47 µm in size with frequent nodding swelling no ascospores were recorded.

Pink rot (*Fusarium oxysporum* Schlechtend ex Fr. f.sp. *cepae* (Hans) Syd and Hans)

The fungus produced micro conidia, macro and Macro conidia were multi septate and hyaline in colour, micro conidia were in abundance.

White rot (*Sclerotium cepivorum*)

The mycelium of the fungus was silky white, hyaline, hyphae, thin, bold, septate. The Sclerotia were light brown to dark brown and resembled to mustard seed (poppy seed) pin head size like.

Materials and Methods

Location of the site

The field investigations were conducted at Garlic Research Experimental Area, Adhartal Tank farm, Department of Plant Breeding & Genetics, JNKVV, Jabalpur.

The investigations were conducted in Garlic crop grown at Jabalpur that lies between 22°21' and 80°58' East longitude at an altitude 411.78 meter above the mean sea level.

Identification of seed associated mycoflora

Association of mycoflora with of garlic clove was determined. The mycoflora that were associated with diseases, were identified based upon the symptoms and structure and fruiting bodies produced either under *in-vitro* and *in-vivo* conditions. The identity of the associated microorganisms was confirmed

through various keys developed by other scientists.

Detection of mycoflora association with garlic cloves and emergence clove

Detection of mycoflora associated with garlic clove was made using standard blotter method and standard paper towel method. Garlic cloves were obtained from 5 districts covering 12 villages. In standard blotter method five garlic clove were place onto of the top of blotter in a plate. In all 100 cloves were tested from each locations. Observations were recorded on the associated mycoflora on 7th days after incubation. The mycoflora were identified on the basis of habit characters and later confirm through slide preparation for fungal structures and spors.

Standard blotter method

In this method, three circular blotter papers of the size of the Petri dish were cut and dipped in sterilized water. Excess water was drained and soaked sheets were placed in each Petri dish. Five cloves were placed in each Petri dish with the help of sterilized forcep under aseptic conditions of inoculation chamber. In the outer circle 4 cloves were placed, and 1 in the inner circle so as to allow in the equal distance between the clove. Cloves plates were kept for the incubation in the chamber. Fungi were identified by making slides and observing under microscope on 7th day of incubation with the help of identification manuals.

Standard paper towel method

Standard paper towel method (ISTA 1996) was used. Clove samples were selected from the harvest of diseased plants. In this method, 100 cloves of varieties were used. The towel (blotter) papers were moistened with sterile

water. Excess of water was removed. The paper were stretched and placed over a clean surface of table on a paper, 10 cloves were arranged on the half portion of the towel paper. Cloves were covered with the other half portion of the paper and rolled over. A wax paper was wrapped on rolled paper towel and both ends were tightened with rubber band, it prevented the runoff water as well as helped in maintaining of humidity require for germination. The rolled towel papers were kept in a slanting position. The cloves towels were placed in clove germination at 25⁰C with relative humidity about 85%. Seedlings were examined on 14th day of incubation and germination percentage was calculated.

Clove treatment

Influence of clove treatment with 3 bio pesticides and 4 chemical fungicides was determined on the emergence of garlic clove, clove rot complex and Stemphylium blight disease under natural field conditions at Jabalpur during Rabi 2015-2016.

Cloves were treated with bio pesticides and fungicides and % emergence was recorded after 15 days after sowing of clove at field and under lab conditions. Clove treatment with *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescense* were @ 10g/kg of clove and for fungicides doses was @3 g/kg of clove was taken. The variety G 50 was selected for clove treatment.

Statistical analysis

Analysis of observations taken on different variables was carried out to know the degree of variation among all the treatments. The pooled data was statistically analyzed through randomized block design. The results were obtained through analysis of variance is given in appendix and the skeleton of analysis of variance (Table.1) given below:

$$SEm_{\pm} = \sqrt{\frac{EMS}{r}}$$
$$SEd_{\pm} = \sqrt{\frac{2EMS}{r}}$$

CD = SEd \pm x t at 5%

Where,

r = No. of replications

EMS = Error mean square

SEm \pm = Standard error of treatment mean

SEd \pm = Standard error of difference to two treatment mean

CD = Critical difference to two treatments mean

Results and Discussion

Influence of clove treatment with bio-pesticides and fungicides

Effect of clove treatment with 3 bio-pesticides and 4 fungicides was determined on emergence and clove rot complex. Cloves of local variety obtained from Neemuch, was treated and sown in the sterilized soil in plastic trays. Observations were recorded after 11 days of planting (sowing) on emergence and development of clove rot complex.

Effect on clove emergence

Under *in-vitro* Condition

Data presented in Table 1.1 indicate that in untreated clove, emergence was 72% whereas in treated clove, increase emergence was recorded. Maximum (88%) emergence was

observed in clove, treated with Carboxin+Thirum (as Vitavax power 200, commercial formulation) @ of 3.0g /kg clove, in bio-pesticides 87 % emergence was recorded in cloves treated with *Trichoderma viride* and *Trichoderma harzianum*. Cloves treated with *Pseudomonas fluorescense* was did not show any increase. Clove treated with Copper oxy chloride used (as blue copper commercial formulation) and Carbendazium + Mancozeb (as Saff commercial formulation) @ of 3.0g/ kg clove was promising and resulted in 86% clove emergence.

Effect on clove rot complex

Data presented in Table-2 indicate that in untreated cloves rotting was recorded due to *Fusarium oxysporum*, *Sclerotium cepivorum*, and *Aspergillus* spp. The association of fungi with rotted cloves was confirmed through microscopic observation. In untreated control clove rot was maximum(23%) whereas least number of cloves were infected in clove treated with Carboxin+Thirum and Carbendazium+ Mancozeb upto (9%).

In clove treated with *Trichoderma viride* and *Trichoderma harzianum* clove rot upto 10% was recorded whereas clove treated with bio-pesticide was not much effective (Table 2)

Effect of fungicide treatment on clove emergence

Under *In-vivo* condition

Data presented in Table 3. indicate that clove emergence was 70% in untreated clove whereas maximum emergence 89% was recorded in clove treated with Carboxin + Thirum and Carbendazium+ Mancozeb @ of 3.0 g/kg clove. As compared to chemical, fungicide, bio-pesticide had showed lesser effect. Clove treatment with *Trichoderma viride* and *Trichoderma harzianum* in clove

emergence was upto 88%. Under field condition untreated clove exhibited higher rotting (25%) which decreased an untreated clove (6.0 to 19.0 %), clove treated with Carboxin+ Thirum resulted in minimum rotting (6.0%) followed by 8% in clove treated with *Trichoderma viride* and *Trichoderma harzianum*. Clove treated with bacterial bio-pesticide was not effective, 19% clove rot observed as compared to untreated control (25%).

Data presented in Table 3 indicate that in untreated clove tender tip blight ranged upto 32% while minimum disease (12%) was in clove treated with Carboxin + Thirum and Carbendazium+ Mancozeb.

Effect on clove associated mycoflora

Effect of bio-pesticide and fungicide on the association of *Alternaria porri*, *Fusarium oxysporum*, *Aspergillus niger* and *Aspergillus flavus* was determined, by using the pre-identified clove sample.

Data presented in Table 4. indicate that when the clove sample with 14% natural infection of *Alternaria porri* was used as in untreated control, The association was reduced in the range of 2.0 to 7.0 %, clove treated with Carboxin + Thirum and Carbendazium + Mancozeb did not show any association of *Alternaria porri*, practically the infection was eliminated. In clove treated with *Trichoderma viride* and *Trichoderma harzianum* exhibited association of *Alternaria porri* upto 4%. Bacterial bio-pesticide, *Pseudomonas fluorescence* was ineffective.

Clove treatment with bio-pesticide and fungicide was effective in reducing the association of mycoflora, however clove treated with bacterial pesticide was not effective. Association of *Fusarium*

oxysporum ranged from 2.0 to 3.0 % in treated clove as compared to 11% in untreated cloves. No association of *Fusarium oxysporum* was recorded in clove treated with Carboxin+ Thirum and Carbendazium+ Mancozeb @ of 3.0g /kg clove.

A similar trend was recorded and in untreated clove association of *Aspergillus niger* and *Aspergillus flavus* no associated with clove treated with Carboxin+ Thirum and Carbendazium+ Mancozeb and copper oxy chloride. Increase sprouting was recorded (92%) in clove treated with Carboxin + Thirum, Carbendazium+ Mancozeb and copper oxy chloride (89 to 92%) as compare to untreated control (83%)(Table 4).

Considering the association of pathogens and comparatively lower sprouting, investigations were made on the possible improvement through clove treatment using bio-pesticides and common commercially available fungicides. Cloves of local variety obtained from Neemuch were treated with 3 bio-pesticides and 4 fungicides to determine the impact on clove emergence and clove rot complex. Maximum (88%) emergence was recorded in clove treated with Carboxin + thirum (as Vitavax power 200) @ 3g/ kg clove. While clove treated with *Trichoderma viride* and *T. harzianum* @ 10g/kg clove resulted in 87% emergence as compared to 72% in untreated cloves. Cloves treated with bacterial biopesticide *Pseudomonas fluorescence* were not effective, while application of Copper oxychloride and Carbendazim + Mancozeb @ 3g/kg were promising with higher emergence. Clove rot complex due to infection of *Fusarium oxysporum*, *Sclerotium cepivorum* and *Aspergillus niger* was observed. Clove treated with Carboxin + thirum or Carbendazim + Mancozeb improved the emergence and reduced the rotting as compared to untreated ones.

Table.1 Analysis of variance skeletable

Sr. No.	Source of variance	Degree of freedom (DF)	Sum of square (SS)	Mean sum of square (MSS)	Calculate d F. value	Table value (5%)
1.	Replications	(r-1)				
2.	Treatments	(t-1)				
3.	Error	(r-1)(t-1)				
	Total	(rt-1)				

Table.2 Influence of clove treatment with bio-pesticide and fungicide on the emergence and incidence of cloves rot complex of garlic under *In-vitro* conditions

Fungicide	Dose per kg clove	Percent clove emergence	Percent clove rot complex
<i>Trichoderma viride</i>	10g	87	10
<i>Trichoderma harzianum</i>	10g	87	10
<i>Pseudomonas fluorescense</i>	10g	82	19
Carboxin + Thirum	03g	88	08
Carbendazim + Mancozeb	03g	86	09
Copper oxy chloride	03g	86	12
Copper hydroxide (as Bordeaux mixture)	01%	85	12
Control (Untreated)	00	72	23
Sem		1.59	1.05
CD 5%		4.84	3.19

Table.3 Influence of clove treatment with bio-pesticide and fungicide on the incidence of basal rot complex, blight and spots of garlic during Rabi 2015-16 at STR field Jabalpur

Fungicide	Dose per kg clove	Percent clove emergence	Percent basal rot complex	Percent tender tip blight
<i>Trichoderma viride</i>	10g	88	08	15
<i>Trichoderma harzianum</i>	10g	86	08	15
<i>Pseudomonas fluorescense</i>	10g	80	19	25
Carboxin + Thirum	03g	89	06	12
Carbendazim + Mancozeb	03g	89	10	12
Copper oxy chloride	03g	86	14	17
Copper hydroxide	01%	85	15	19
Control (Untreated)	00	70	25	32
Sem	-	1.16	1.00	1.01
CD 5%	-	3.53	3.04	3.09

Table.4 Influence of cloves treatment with bio-pesticide and fungicide on the association of mycoflora as tested by Standard blotter method (ISTA1996)

Fungicide	Dose per kg clove	Percent association of mycoflora			Percent sprouting		
<i>Trichoderma viride</i>	10g	03	03	03	85	85	86
<i>Trichoderma harzianum</i>	10g	04	03	03	85	85	89
<i>Pseudomonas fluorescense</i>	10g	07	09	08	80	79	73
Carboxin + Thirum	03g	00	00	00	92	93	92
Carbendazim + Mancozeb	03g	00	00	00	92	92	93
Copper oxy chloride	03g	04	00	00	89	89	89
Copper hydroxide (as Bordeaux mixture)	01%	02	02	05	85	88	89
Control (Untreated)	000	-	-	-	83	83	83
Initial infection of <i>Alternariaporri</i>		14	-	-	14	-	-
<i>Fusarium oxysporum</i>		-	11	-	-	11	-
<i>Aspergillus niger</i> and <i>Aspergillus flavus</i>		-	-	30	-	-	30
SEM		0.72			1.08		
CD 5%		2.22			3.28		

The studies were recorded in sterile soil conditions under lab conditions and natural field conditions.

Under lab conditions, using sterile soil, minimum clove rot complex was noticed in cloves treated with Carboxin + thirum (8%) as compared to untreated (23%) with higher clove emergence in treated (88%) and lower in untreated (72%), respectively. Similar trend was recorded under natural field conditions with higher emergence (89%) and lower clove rot (6%) in Carboxin + thirum treated as compared to untreated cloves, emergence (70%) and rotting (25%), respectively.

For the management of plant diseases different fungal and bacterial microbes have been used with proved antimicrobial effects (Cook and Baker, 1988). Application of microbial antagonist has shown a promising ecofriendly impacts as compared to chemicals. A number of research groups are

investigating multiple approaches to control diseases. Different species of *Trichoderma* have successfully used and promising results have been recorded for controlling garlic diseases (Metcalf *et al.*, 2004; Sharifi *et al.*, 2010; Francisco *et al.*, 2011; Kakvan *et al.*, 2013; Khiyami *et al.*, 2014). Gupta *et al.*, (2011) and Ahmad and Tribe (1977) have noticed the efficacy of *Trichoderma viride*, *Gliocladium zeae* and *Coniothyrium minitans*.

In dual culture method, efficacy of *Trichoderma viride* has been advocated against *Fusarium oxysporum* f.sp. *cepae* (Riker and Riker, 1936; Dennis and Webster, 1971), against *Sclerotium cepivorum* (Rini and Sulochana, 2007; Akrami *et al.*, 2009; Naraghi *et al.*, 2013). Combinations of bio-pesticide and fungicide have also been attempted for the management of *Fusarium* sp., *Sclerotium* sp. affecting garlic crop. Application of Tebuconazole and *Trichoderma hamatum* has provided good

control against *Sclerotium cepivorum*. Koriema *et al.*, (1991) reported the efficacy of application of benomyl, carbendazim, maneb, thirum, and carboxin hydroxyquinoline against *Fusarium oxysporum* f.sp. *cepae*.

Reduced incidence of tender tip blight was also recorded in the present investigation by the application of chemicals and bio-pesticides, apart from improvement in emergence and reduction in clove rot complex, incited by *Fusarium oxysporum* and *Sclerotium cepivorum*. Srivastava *et al.*, (1995) and Gupta *et al.*, (1996) reported the efficacy of chlorothalonil, difenconazole, thiophanate methyl, penconazole and hexaconazole against *Stemphylium vesicarium*. Jakhar *et al.*, (1996) demonstrated the effectiveness of mancozeb and copper oxychloride whereas Hug *et al.*, (1994) proved the efficacy of carbendazim+ mancozeb, tebuconazole, propiconazole against *Stemphylium* blight.

Clove seed associated mycoflora were successfully managed with the application of Carbendazim + Mancozeb @ 0.3% and Carboxin + thirum @ 0.3% during the present study. Clove sample with known infection of *Alternaria porri*, *Fusarium oxysporum* and *Aspergillus niger* and *Aspergillus flavus* were selected. Clove treatment with fungicide reduced association of these mycoflora. Untreated cloves served as check.

Clove treatment with Carboxin + thirum @ 3g/kg or Carbendazim + Mancozeb @ 3g/kg clove were effective for improving the sprouting, emergence, under lab and field conditions. The combination also reduced the clove rot complex and incidence of *Stemphylium* tender tip blight. Clove treatment with fungal bio-pesticides (*Trichoderma viride*) was effective as

compared to bacterial bio-pesticides (*Pseudomonas fluorescens*). Clove rot reduced due to the application of chemical fungicides.

Infection of *Aspergillus* spp., *Fusarium* spp. and *Sclerotium* sp. revealed in clove rot complex, that could be managed by clove treatment with Carboxin + thirum @3g/kg or Carbendazim + Mancozeb @ 3g/kg clove.

Clove treatment with *Trichoderma viride* @10g/kg clove resulted in higher emergence.

Reduced incidence of tender tip blight was noticed due to clove treatment with fungal bio-pesticides and fungicides as compared to bacterial bio-pesticides.

References

- Ahlawat Y S. 1974. A mosaic disease of garlic in Darjeeling hills. *Science & Culture* 40: 466-467.
- Ahmad A H M and Tribe H T. 1977. Biological control of white rot of onion (*Sclerotium cepivorum*) by *Coniothyrium minitans*. *Plant Pathology* 26: 75-78.
- Ahmad S and Karimullah. 1998. Relevance of management practices to downy mildew in onion. *Sarhad Journal of Agriculture* 14: 161-162.
- Akrami M, Ibrahimov A, Zafari D M and Valizadeh E. 200. Control of *Fusarium* rot of bean by combination by *Trichoderma harzianum* and *Trichoderma asperellum* in greenhouse condition. *Agricultural Journal* 4: 121-123.
- Apaza W E and Matos L. 2000. Reaction of onion cultivars to basal plate rot caused by *Fusarium oxysporum* f.sp. *cepae*. *Fitopatologia* 35: 231-236.
- Crowe F J and Hall D H. 1980. Soil temperature and moisture effects on

- Sclerotia* germination and infection of onion seedlings by *Sclerotium cepivorum*. *Phytopathology* 70: 74- 78.
- Dennis C and Webster J. 1971a. Antagonistic properties of species groups of *Trichoderma* I. Production of non-volatile antibiotics. *Transactions British Mycology Society* 57: 25-39.
- Deresse D. 2010. Antibacterial effect of garlic (*Allium sativum*) on *Staphylococcus aureus*. An in vitro study. *Asian Journal of Medical Sciences* 2(2):62-65.
- Dhiman J S, Chadha M L and Sidhu A S. 1986. Studies on the reaction of onion genotypes against purple blotch. *Vegetable Science* 13: 304-309.
- Everts K L and Lacy M L. 1990. The influence of dew duration, relative humidity and leaf senescence on conidial formation and infection of onion by *Alternaria porri*. *Phytopathology* 80: 1203-1207.
- Fenwick G R and Hanley A B. 1985. The genus *Allium*. Part 3. Medicinal effect. *CRC critical Review Food Science & Nutrition* 1: 1-74.
- Francisco D H, Angelica M P, Gabriel M, Melchor C S, Raul R, Cristobal N and Francisco CR. 2011. In vitro antagonist action of *Trichoderma* strains against *Sclerotium sclerotiorum* and *Sclerotium cepivorum*. *American Journal of Agriculture Biology Science* 6 (3): 410-417.
- Gupta R C, Srivastava K J and Gupta R P. 2011. Management of economically important soil borne diseases in onion bulb crop. National symposium on vegetable biodiversity held at JNKVV, Jabalpur 87p.
- Gupta R P, Srivastava P K and Sharma R C. 1996. Chemical control of purple blotch and *Stemphylium* diseases of onion. Newsletter National Horticultural Research and Development Foundation, 16: 14-16.
- Hug M L, Malakar P K and Nahar M S. 1994. Chemical control of purple blotch disease of onion seed crop Bangladesh Journal Scientific Industries Research 29: 157-161.
- Jakhar S S, Duhan J C and Suhag L S. 1996. Studies on the epidemiology and survival of *Stemphylium vesicarium* (Wallr.) Simmons in debris and seeds of onion Seed Research 24: 135-140.
- Kakvan N, Heydari A, Zamanizadeh H R, Rezaee S and Nraghi L. 2013. Development of new bioformulations using *Trichoderma* and *Talaromyces* fungal antagonists for biological control of sugar beet damping-off disease. *Crop Protection* 53 (1): 80-84.
- Khiyami M A, Almoammar H, Awad Y M, Alghuthaymi MA Abd- Elsalsm K A. 2014. Plant pathogen nanodignostic techniques forthcoming changes. *Biotechnology Equipment*.28: 775-785
- Mathur B L and Sankhala H C.1963. Basal rot of onion. *Current Science* 31:40.
- Mathur K and Shukla S N. 1963. Evaluation of fungicides against *Alternaria porri* and *Stemphylium vesicarium* disease of onion in Rajasthan. *Journal Mycology Plant Pathology* 36(2): 323-324.
- Metcalf D A, Dennis J J C and Wilson C R. 2004. Effect of inoculums density of *Sclerotium cepivorum* on the ability of *Trichoderma koningii* to suppress white rot of onion. *Plant Disease Research* 88 (3): 287-291.
- Naraghi L, Heydari A, Rezaee S, Razavi M and Afshari-Azad H. 2013b. Promotion of growth characteristics in greenhouse cucumber and tomato by *Talaromyces flavus*. *Intranational Journal Agriculture Science Research* 2 (3): 129-141
- Quadri S M H, Shrivastava K S, Bhonde S R, Pandey U B and Bhagchandani P M. 1982. Fungicidal bioassay against some important pathogens of onion.

- Pesticides 16: 11-16.
- Rai B, Agrawal M, Agrawal S B. 1976. Threat to food security under current levels of ground level ozone: a case study for Indian cultivars of rice. *Atmospheric Environment* 44: 4272-4282.
- Reuter H D, Koch H P and Lawson D L. 1996. Therapeutic effects and application of garlic and its preparations In: Koch HP and Lawson DL (eds) *Garlic. The science and therapeutic applications of Allium sativum L. and related species* Second edition 135-21p.
- Riker A J, Riker P B. 1935. Bacteria pathogenic on plants. In *The Genetics of Pathogenic Organisms* Publication American Association Advancement Science, 12: 46-56.
- Rini C R and Sulochana K K. 2007. Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. *Journal Tropical Agriculture* 45: 21–28.
- Sandhu K S and Kang M S. 1988. Occurrence of rust on garlic in Punjab. *Plant Disease Research* 3(1): 50
- Schwartz H F and Mohan S K. 1996. *Compendium of onion and garlic diseases*. American Phytopathological Society St. Paul Minnesota USA.
- Scott M R. 1956. Studies on the biology of *seclerotium cepivorum* Berk. Growth of mycelium in soil. *Annals of Applied Biology* 44: 576-583.
- Sharifi K and Zare R. 2010. Investigation on genetics diversity of *Fusarium oxysporum* causing potato Fusarium wilt by pathogenicity testes and RAPD markers. *Iranian Journal of Plant Pathology* 45: 9-25.
- Sharifi R, Ahmadzadeh M, and Talebi-Jahromi K. 2010. Pyoverdine production in *Pseudomonas fluorescens* UTPF5 and its association with suppression of common bean damping off caused by *Rhizoctonia solani* *Journal of Plant Protection Research* 50 (1): 72–78.
- Shrivastava K J, Bhardwaj B S and Gupta R P. 1992. Management of garlic diseases in India. *NHRDF Newsletter* 15(3): 6-9.
- Shrivastava K J, Bhardwaj B S and Gupta R P. 1992. Management of garlic diseases in India. *NHRDF Newsletter* 15(3): 6-9.
- Singh B M and Sharma Y R. 1977. Occurrence of leaf blight of garlic caused by *Stemphylium botryosum* in India. *Indian Phytopathology* 30: 272-273.
- Singh B M, Singh D and Paul Y S. 1987. Outbreak of downy mildew of onion in Himachal Pradesh. *Plant Disease Reporter* 2: 124.
- Sovová M and Sova P. 2004. Pharmaceutical importance of *Allium sativum* L. Hypolipidemic effects in vitro and in vivo. *Česká a slovenská farmacie*, 53: 117–123.
- Thind T S, Sharma R C and Jhooty J S. 1985. Occurrence of two fungal diseases of onion in Punjab. *Indian Journal of Mycology and Plant Pathology* 15: 90.
- Walker J C and Tims E C. 1924. A *Fusarium* bulb rot of onion and the relation of environment to its development. *Journal Agriculture Research* 28: 683-693.
- Walker J C. 1952. *Diseases of Vegetables Crops*. Mc Graw Hill Rock Company Incorporation New York 529p.
- Walker J C. 1952. *Diseases of Vegetables Crops*. Mc Graw Hill Rock Company Incorporation New York 529p.