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Efficacy of different phyto-extracts and bio-control agents against foliar blight complex of onion *in vitro*

KK Gondaliya, SI Patel and NK Thumbadiya

Abstract
Onion (*Allium cepa* L.) is one of the most widely used vegetable due to its flavouring and seasoning the underground vegetable, both at mature and immature bulb stage in tropical and sub tropical countries. The foliar blight complex of onion resulted due to combined infection of *Alternaria porri* and *Stemphylium vesicarium*. The investigation on the fungitoxic effects of widely utilized rhizome, clove, bulb, seed and leaf extracts of various plants belonging to different families against the combined radial growth of *A. porri* and *S. vesicarium* revealed that Ginger extract recorded 83.70 and 86.85% while, garlic extract resulted in 79.81 and 82.04% growth inhibition at 10 and 20% concentration, respectively. The *in vitro* efficacy of various isolates of bio-agents indicated that *Trichoderma viride* (Junagadh) was highly efficacious followed by *T. harzianum* (Junagadh), *T. viride* (Sardarkrushinagar), *T. harzianum* (Sardarkrushinagar), *T. viride* (Navsari), *T. longibrachiatum*, *P. fluorescens* and *Bacillus subtilis.*

Keywords: Phyto-extracts, bio-control agents, foliar blight complex

Introduction
Onion (*Allium cepa* L.) rightly called as "queen of kitchen" is one of the oldest and an important spice, cool season and sensitive to photoperiod crop grown in India as well as tropical and sub tropical countries in the world. The edible portion is formed from swollen leaf sheathes derive from bladed leaves and the inner ones are bulb scales which is known as bulb and develop underground (Brewster, 1994) [6]. On the basis of skin colour there are three types of onion i.e., Red, Yellow and White. The red colour of onion is due to pigment 'Anthocyanin' and yellow colour is due to 'Quercertin' pigment. A volatile oil known as "Allyl-propyl di-sulphide" is the main ingredient responsible for pungency and flavor in bulbs, which help to prevent cancer and acts as a gastric stimulant and promotes digestion (Yawalkar, 1992) [12]. Chopping an onion causes damage to cells which allows enzymes called aliinases to break down amino acid sulfoxides and generate sulfenic acids through lachrymatory factor synthase (LFS) and giving volatile gas known as the onion lachrymatory factor or LF. Tear glands produces tears in order to dilute and flush out the irritant (Imai *et al*., 2002) [9]. The productivity of the onion in India is low as compared to many other countries. This is mainly due to many fungal, bacterial and viral diseases. They are responsible for limiting the production and productivity of onion. Among the foliar diseases, *Stemphylium vesicarium*, the causal agent of white blotch of onion are being considered as an organism involved indirectly with the development of purple blotch (*Alternaria porri*) of onion. It is considered that *S. vesicarium* initiate the infection, which facilitates subsequent infection of *A. porri* causing purple blotch and hence the disease is designated as foliar blight complex (Zakirul, 2013) [13]. The hazardous effects of chemicals used in plant disease management are well known. Further, the potential threat of residues of chemicals leads to health related issues. Hence, an alternative methods like use of phyto-extracts and/or bio-agents is of prime importance. Therefore, present investigation on efficacy of various phyto-extracts and bio-agents against combined growth of *A. porri* and *S. vesicarium* causing leaf blight complex of onion was carried out.

Material and Methods
Collection of diseased samples
The diseased samples of onion showing typical leaf blight symptoms were collected from Horticultural farm, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar Gujarat, India. The infected samples were brought to the laboratory and subjected for tissue isolation on to sterilized Potato dextrose agar (PDA) medium in Petri-plates. The Petri-plates were incubated at temperature 25 + 2 ºC for seven days.

The culture was purified through dilution method and hyphal tip method.

Preparation of phyto-extracts

The effect of thirteen phyto-extracts of different plants belonging to different families were evaluated against combined growth of *A. porri* and *S. vesicarium* under *in vitro* (Ansari, 1995) [1] with 10 and 20 per cent concentration. The fresh plant materials were separately ground in sterilized distilled water at the rate of one ml/g of the plant parts in a sterilized pestle and mortar. The extract were first filtered through two layer of muslin cloth and subsequently filtered through Whatman No. 1 filter paper. This formed the standard plant extract solution (100%). The extracts were centrifuged at the rate of 6000 RPM at 4 °C for 10 minutes. Tenml of the plant extracts were added to 90 ml of the sterilized warm potato dextrose agar medium for 10 per cent concentration and 20 ml of the plant extracts were added to 80 ml of the sterilized warm potato dextrose agar medium for 20 per cent concentration. The medium were poured in to the sterilized Petri-plates under aseptic conditions.

Dual culture method for bio-agents

Different antagonists were tried *in vitro* to test the antagonistic activity against combined growth of *A. porri* and *S. vesicarium* by dual culture method (Dennis and Webster, 1971) [8].

Various bio-agents and pathogens were grown separately on PDA. Sterilized PDA (20 ml) was poured aseptically in 90 mm diameter sterilized Petri-plate. Mycelial disc (7 mm diameter) from seven days old actively growing culture of bio-agents and the associated pathogens were cut aseptically from the periphery of the colony with the help of sterilized cork-borer and placed on solidified PDA approximately 70 mm away from each other. Test pathogens were subjected alone for growth and comparison.

The experiment was conducted using completely randomized design (CRD) and data were statistically analysed using Duncan's New Multiple Range Test. Colony diameter was measured along the two diagonals passing through the colony by excluding the initial diameter (7 mm) of bit. Colony diameter was measured when the control treatment with pathogen reached full growth. The per cent growth inhibition of the fungus in each treatment in comparison with control was calculated by the following equation (Bliss, 1934) [4].

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

Where,

PGI = Per cent growth inhibition,

C = Colony diameter (mm) in control

T = Colony diameter (mm) in treatment

Results and Discussion

Efficacy of different phyto-extracts against the associated pathogen(s) *in vitro*

This information is certainly useful in exploiting inhibitory principle for developing botanical fungicides in plant disease management.

Effect of phyto-extracts

The rhizome, clove, bulb, seed and leafextracts of various plants were evaluated and found inhibitory to the combined radial growth of *A. porri* and *S. vesicarium*. The highest radial growth inhibition (85.28%) was recorded with Ginger (*Zingiber officinale* Rose) rhizome extract which was significantly superior to rest of the phyto-extract. This was closely followed by garlic (*Allium sativum* L.) clove extract (80.93%). The extracts of *datura*, *parthanium*, *neem* leaf and olive also recorded more than 50% growth inhibition of test pathogens.

Effect of concentration

Irrespective of the rhizome, clove, bulb, seed and leaf extracts, the mean inhibitory effect was recorded significantly higher (57.88%) at 20 per cent concentration. The 10% concentration recorded 53.63per cent growth inhibition of *A. porri* and *S. vesicarium*.

Interaction effect of phyto-extract × concentration

The results presented in Table 1 revealed that all rhizome, clove, bulb, seed and leaf extracts at 10 and 20 per cent concentration inhibited the combined growth of both the pathogens significantly as compared to control. Ginger rhizome extract recorded 86.85 and 83.70 per cent growth inhibition of at 20 and 10 per cent concentrations, respectively. The next effective extract was garlic clove which recorded 82.04 and 79.81 per cent radial growth inhibition at 20 and 10 per cent concentration, respectively. Onion bulb extract was observed least effective in inhibiting the combined mycelial growth of *A. porri* and *S. Vesicarium* at both concentrations (31.30% and 33.15%, respectively). Among leaf extracts highest growth inhibition was revealed by *Datura* leaf extract with 77.41 and 72.96% radial growth inhibition at 20 and 10 per cent concentrations, respectively. This was followed by Parthenium leaf extract which recorded 76.30 and 69.07 per cent growth inhibition and was at par with *Datura* leaf extract at 20 and 10 per cent concentration. The next effective phyto-extracts at 10 and 20 per cent concentration in order of inhibition were neem leaf extract (65.37 and 68.52%), olive leaf extract (57.59 and 62.59%), castor leaf extract (50.74 and 55.56%), *tulsi* leaf extract (43.15 and 52.78%), neem seed kernel extract (40.93 and 44.07%), *Nilgiri* leaves extract (38.42 and 40.91%) and Lantana leaves extract (37.21 and39.29%). *Ardusi* leaves extract recorded least growth inhibition of 31.11 and 28.33% at 20 and 10 per cent concentrations, respectively.

Table 1: Growth inhibition of combined culture of *A. porri* and *S. vesicarium* by phyto-extracts at different concentration *in vitro*

Sr. No.	Name of plants	Growth inhibition (%)* Concentration (%)		Mean
		10	20	
1	Ardusi	32.15**^{p} (28.33)	33.88^{op} (31.11)	33.01^{j} (29.72)
2	Datura	58.72^{e} (72.96)	61.62^{de} (77.41)	60.17^{c} (75.19)
3	Neem (Leaf)	53.94^{fg} (65.37)	55.86^{f} (68.52)	54.90^{d} (66.95)
4	Neem (Kernal)	39.75^{q} (40.93)	41.58^{k} (44.07)	40.66^{h} (42.50)
5	Castor	45.41^{j} (50.74)	48.17^{hi} (55.56)	46.79^{f} (53.15)
6	Tulsi	41.04^{k} (43.15)	46.57^{ij} (52.78)	43.81^{g} (44.01)
7	Nilgiri	38.13^{lm} (38.15)	40.61^{kl} (42.41)	39.37^{hi} (40.28)

8	Lantana	36.91^{mn} (36.11)	38.98^{klm} (39.63)	37.96^{i} (37.87)
9	Onion (Bulb)	34.00^{op} (31.30)	35.14^{no} (33.15)	34.57^{j} (32.23)
10	Garlic(Clove)	63.28^{cd} (79.81)	64.91b^{c} (82.04)	64.10^{b} (80.93)
11	Ginger(Rhizome)	66.18^{b} (83.70)	68.72^{a} (86.85)	67.45^{a} (85.28)
12	Olive	49.35^{h} (57.59)	52.30^{g} (62.59)	50.83^{e} (60.09)
13	Parthenium	56.20^{f} (69.07)	60.85^{de} (76.30)	58.52^{c} (72.69)
	Mean	47.54 (53.63)	49.72 (57.88)	-
		Phyto-extract	Concentration	Phyto-extract × concentration
S.Em. ±		0.55	0.22	0.78
C.V.%		2.78		

*Average of three observations;
** Arc-sin transformed values
Figures in parentheses are original values;
Treatment means with the letter(s) in common are not significant by Duncan's New Multiple Range test at 5 per cent level of significance

The strong inhibition potential of ginger is attributed to the fact that it contains over 400 different compounds, a mixture of both volatile and non-volatile chemical constituents such as zingerone, shogaols and gingerols, sesquiterpenoids(β-sesquiphellandrene, bisabolene and farnesene) and a small monoterpenoid fraction(β-phelladrene, cineol and citral). The main constituents of the garlic essential oils are diallylmonosulfide, diallyldisulfide (DADS), diallyltrisulfide and diallyltetrasulfideas describedby Casella *et al*. (2013) [7].

Mishra and Gupta (2012) [11] revealed that *Allium sativum* extracts resulted in 57.31 per cent inhibition of combined mycelial growth of *A. porri*and *S. vesicarium*at 10 per cent concentration. Brahmane (2015) [5] elucidated that *Zingiber officinale* and *Allium sativum* extracts recorded least purple blotch severity of 32.18 and 25.22%, respectively. At 15 per cent concentration, garlic extracts resulted in 65.37 and 57.42% inhibition in mycelial growth of *A. porri* as described by Arunkumara *et al*. (2016) [2] and Jhala *et al*. (2017) [10], respectively.

Thus, the results of inhibitory effects of phyto-extracts against *A. porri* and *S. vesicarium* obtained in present study are in accordance with earlier reports.

Efficacy of promising bio-agents against associated pathogen(s) *in vitro*

Isolates of *Trichoderma* spp. are well documented as effective bio-control agent in managing many pathogens. However, inadequate information on the performance of the antagonists under varying conditions is a major constraint in large scale adoption of this technology in general.

Isolates of *Trichoderma* spp. are well documented as effective bio-control agent in managing many pathogens. However, inadequate information on the performance of the antagonists under varying conditions is a major constraint in large scale adoption of this technology in general.

In the present study, different known bio-agents were evaluated for their antagonistic effect against the radial growth of *A. porri* and *S. vesicarium*. The data (Table 2) clearly revealed that all the bio agents in general are quite efficacious against combined growth of *A. porri* and *S. vesicarium in vitro.* The inhibition of the combined mycelial growth was ranged 35.90 to 61.28 per cent.

Out of eight antagonists tested, maximum inhibition (61.28%) of the combined radial growth of *A. porri* and *S. vesicarium* was recorded in *T. viride* (Junagadh) which was at par with *T. harzianum* (Junagadh) and both were significantly superior to rest of the bio-agents. This was followed by *T. viride* (Sardarkrushinagar) and *T. harzianum* (Sardarkrushinagar) with respective growth inhibition of 53.59 and 50.77%, respectively. Among the various *Trichoderma* spp., *T. longibrachiatum* was proved comparatively less effective with only 36.15% growth inhibition. The mean data (fig. 4.6) on efficacy of various *Trichoderma* spp. clearly indicated that *T. harzianum* and *T. viride* are more efficacious against the combined radial growth of *A. porri* and *S. vesicarium.*

Further, and attempt was also made to compare the efficacy of fungal and bacterial bio-agents. The mean data revealed that fungal bio-agents are more effective with mean growth inhibition of 51.54% compared to bacterial bio-agents. Where mean growth inhibition was recorded only 36.54% (Fig. 1)

Table 2: Growth inhibition of combined culture of *A. porri* and *S. vesicarium* by bio-agents *in vitro*

Sr. No.	Bio-agents	Growth of Pathogen (mm)	Growth inhibition (%)
1	*Trichoderma harzianum* (Sardarkrushinagar)	32.00	45.42**^{bc}(50.77)
2	*Trichoderma harzianum* (Junagadh)	26.50	50.30^{a}(59.23)
3	*Trichoderma viride* (Sardarkrushinagar)	30.17	47.04^{b}(53.59)
4	*Trichoderma viride* (Junagadh)	25.17	51.50^{a}(61.28)
5	*Trichoderma viride* (Navsari)	33.67	43.95^{c}(48.21)
6	*Trichoderma longibrachyatum*	41.50	36.94^{d}(36.15)
7	*Pseudomonas fluorescens*	40.83	37.55^{d}(37.18)
8	*Bacillus subtilis*	41.67	36.79^{d}(35.90)
9	Control	65.00	-
S.Em.±		0.58	
C.V.%		2.29	

*Average of three replications; **Arc sin transformed values; Figures in parentheses are original values; Treatment means with the letter(s) in common are not significant by Duncans New Multiple Range test at 5 percent level of significance

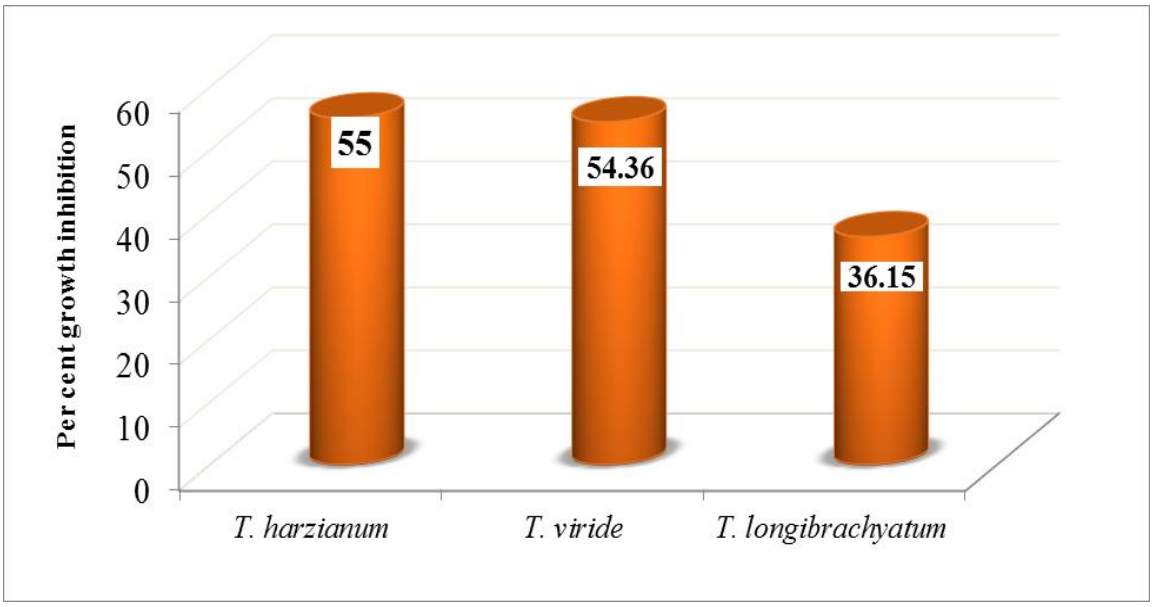

Fig 1: Efficacy of *Trichoderma* spp. against *A. porri* and *S. vesicarium*

Similar finding were also reported by earlier research workers. Mishra and Gupta (2012) [11] evaluated the efficacy of eight bio-agents and revealed that *T. viride* was the most effective in inhibition of mycelial growth (53.17 and 56.15%) followed by *T. harzianum* (53.17 and 51.95%) and *T. koningii* (46.65 and 45.25%) of *A. porri* and *S. vesicarium*, respectively. Different four fungal bio-agents *viz*., *T. harzianum, T. viride, T. virens, T. konnigii*were evaluated *in vitro* condition against *A. porri* by Arunkumara *et al*. (2016) [2]. Among these, *T. harzianum* (54.00%) recorded the maximum inhibition of mycelial growth of *A. porri* followed by *T. viride* (52.25%). They also reported that *B. subtilis* and *P. fluorescens* were least effective with 31.50 and 20.25% growth inhibition, respectively. The antagonistic effect of *Trichoderma viride* against *Alternaria porri* revealed strong antagonism with 85.45% growth inhibition (Bhandekar *et al*., 2019) [3].

The results of the present investigations also revealed the superiority of bio-agents *viz*., *Trichoderma viride* and *T. harzianum* as reported by earlier research workers. Thus, results of the present study are in agreement with the earlier reports.

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