Int.J.Curr.Microbiol.App.Sci (2018) *7*(7): 3752-3757

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 07 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.707.434

Efficacy of Some Fungicides and Bio-control Agents against Tuber Rot (*Fusarium oxysporum*) of Kalazeera (*Bunium persicum*)

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Keywords

Bio-control, Kala zeera, *Fusarium oxysporum*, *In-vitro*, Non- systemic fungicides, Systemic fungicides

Article Info

Accepted:
26 June 2018
Available Online:
10 July 2018

A B S T R A C T

Six non-systemic fungicides *viz*., captan 50 WP, copper oxychloride 50 WP, dodine 65 WP, mancozeb75 WP, propineb 70 WP and zineb 80 WP and five systemic fungicides *viz*., bitertanol 25 WP, carbendazim 50 WP, difenconozole 25 EC, hexaconozole 5EC, and myclobutanil 10 WP were evaluated against tuber rot of kalazeera caused by *Fusarium solani*. The *in-vitro* evaluation of non-systemic fungicides through poisoned food technique at five different concentrations *viz*., 50, 100, 250, 500 and 1000 ppm on active ingredient basis (a.i) indicated that dodine was most effective exhibiting mean mycelial growth inhibition of 82.27 per cent followed by captan (67.99%). Copper oxychloride proved least effective and resulted in only 29.81 per cent mean inhibition of mycelial growth. Among the systemic fungicides, evaluated at concentrations *viz*., 25, 50, 100, 200 and 500ppm on active ingredient basis (a.i), carbendazim proved most effective exhibiting mean mycelial growth inhibition of 90.23 per cent followed by hexaconozole and bitertanol with 71.24 and 64.56 per cent mean mycelial growth inhibition, respectively. Myclobutanil proved least effective and resulted in only 52.76 per cent mean inhibition of mycelial growth. Five strains of *Trichoderma viride* strains designated as Tv-1, Tv-2 Tv-3, Tv-4, Tv-5 and two strain of *Trichoderma harzianum* designated as Th-1 and Th-2 were also evaluated against tuber rot pathogen. Among the bio-control agents Tv-4 was most efficacious and resulted in 48.20 mean mycelial growth inhibition followed by Tv-2 with 47.06 per cent mean inhibition of mycelial growth. *Trichodermaviride*-5 (Tv-5) proved least efficious and resulted in only 26.40 per cent mean inhibition of mean mycelial growth.

Introduction

Kala zeera (*Bunium persicum)* also black cumin is an economically important culinary crop that is cultivated for its seed pods and its tuber like roots. In India, kalazeera is cultivated in high altitude regions of Himachal Pradesh and Jammu and Kashmir. In Jammu and Kashmir, Kala zeera is confined to hilly traits of Gurez, Machill, Tangdar, Pulwama, Paddar, Karnah, Karewas of Budgam and Srinagar. As per the Department of forest estimates for year 1996- 97, Kala zeera occupied about 225 hectares of forestland.

Since the crop usually grows wild in scattered pockets, the yields are lower at 129 kg/ha (J&K) and 179 kg/ha (H.P), as compared to 350-400 kg/ha in the case of caraway (Panwar, 1992). The Yield of kalazeera is dwindling year after year due to the diseases, insect pest, inadequate planting density, high weeds incidence lack of nutritional processing techniques and poor crop management practices (Anonymous 2004). Kala zeera is prone to the number of diseases, including both fungal and bacterial. Under temperate conditions of Kashmir kalazeera is attacked by *Alternaria* leaf spot and *Cercospora* leaf spot pathogens but tuber rot caused by *Fusarium oxysporum* is most important disease of kalazeera which causes drying of foliage and rotting of tubers. White fungal mycelia can be observed on the tubers at the late stages of disease development. Incidence of this disease has been reported to vary from 80 to 90 per cent and yield loss of 50 to 60 per cent has been reported (Badri *et al*., 2013). The use of fungicides and biological methods are most effective andreliable methods of controlling the disease. Fungicides with novel chemistry are being introduced and evaluated before their application can be recommended to farmers. Therefore, laboratory evaluation of the fungicides is of paramount importance. There is constant need to watch and evaluate new fungicides along with some non-chemical method of controlling the wilt disease (Patel, 1998). Therefore, present studies were carried out to evaluate non- systemic and systemic fungicides and some bio-control agents against the disease and the results are presented herein.

Materials and Methods

Sixnon-systemic fungicides *viz*., captan 50WP, copper oxychloride 50 WP, dodine 65 WP, mancozeb 75 WP, propineb 70WP and zineb 80WP and five systemic fungicides *viz*., bitertanol 25WP, carbendazim 50 WP, difenconazole 25EC, hexaconozole 5EC, and myclobutanil 10WP were evaluated *in-vitro* on active ingredient (a.i) basis against tuber rot of kalazeera caused by *Fusarium solani*t hrough poisoned food technique (Carpenter, 1942) using potato dextrose agar medium. The non-systemic fungicides were evaluated at 50, 100, 250, 500 and 1000 ppm and systemic fungicides at 50, 100, 200, 400 and 500 ppm. The required concentrations of fungicides were prepared by adding appropriate amount of fungicides to sterilized molten PDA medium in conical flasks. Thirty millilitre of such amended PDA was aseptically poured in sterilized Petri plates. A 5 mm diameter mycelial disc of FOC was aseptically placed in the centre of each Petri plate. Petri plates containing PDA media amended with equal amount of sterilized distilled water and inoculated with mycelial disc of *Fusarium oxysporum* (5 mm diameter) served as check. Each treatment was replicated thrice and incubated at 28±2^{o}C for 48 hrs. The comparative efficacy of fungicides was calculated as per cent inhibition of mycelial growth of the test fungus in each treatment as compared to check by the following formula:

Per cent mycelial growth inhibition =

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

Where

C = Radial mycelial growth (mm) in check

T = Radial mycelial growth in the treatment (mm)

In-vitro evaluation of bio-control agents

The biological control agents were isolated from soils of different parts of Kashmir and designated as *Trichoderma viridi 1*, *Trichoderma viridi 2, Trichoderma viridi* 3, *Trichoderma viridi* 4 and *Trichoderma viridi*

5, and *Trichoderma harzianum* 1 and *Trichoderma harzianum* 2. The pure culture was multiplied in the test tubes slants. Agar slants of these cultures were placed in refrigerator at 4^0C. Mycelial discs (5mm diameter)of bio-control agent and the pathogen were simultaneously inoculated at the opposite ends of the Petriplates, containing about 20 ml of PDA medium. Three Petri plates were used for each biological control agent and the same number was kept as control. Inoculated Petri plates were incubated at $25^{\pm}2^{o}$C for 7-10 days. The data regarding per cent inhibition of mycelial growth were calculated by standard method.

Results and Discussion

Persual of data (Table 1) indicate that among non-systemic fungicides, dodine was most effective exhibiting highest mean mycelial growth inhibition of 82.27per cent followed by captan (67.92%)while mancozeb, zineb and antracol resulted in mean inhibition of mycelial growth of 58.98, 46.86 and 46.14 per cent, respectively. Copper oxychloride proved least effective among non-systemic fungicides and resulted in lowest mean mycelial inhibition of 29.81per cent only. In general, the efficacy of fungicides increased with increase in their concentrations. At the lowest concentration of 50 ppm, dodine was most effective causing 61.66 per cent mean mycelial inhibition followed by captan (44.40) while copper oxychloride was least efficacious (25.90%). Similar trend was observed with increase in concentration and at the highest concentration (1000 ppm) dodine completely inhibited the mycelial growth of the pathogen followed by captan which caused 81.43 per cent mean mycelial inhibition and copper oxychloride resulted in lowest mean mycelial inhibition of only 38.20 per cent.

Among the five systemic fungicides (Table 2), carbendazim proved most effective exhibiting highest mean mycelial growth inhibition of 90.23per cent followed by hexaconozole and bitertanol with 71.24 and 64.56 per cent mean mycelial growth inhibition, respectively. Difenoconazole resulted in mean mycelia growth inhibition of 60.84per cent, while myclobutanil proved least effective among the systemic fungicides and resulted in lowest mean mycelial growth inhibition of only 52.76per cent mean.

In general, the efficacy of fungicides increased with increase in their concentrations. At the lowest concentration of 50 ppm, carbendazim was most effective causing 82.00 per cent mean mycelial inhibition followed by hexaconazole (53.80%) while myclobutanil was least efficacious (43.53%).

Similar trend was observed with increase in concentration of fungicides and efficiency of fungicides increased with increase in their concentrations. Carbendazim resulted in100 per cent inhibition at 200 and 500 ppm concentrations. However, no other fungicide could cause complete inhibition of mycelial growth of the pathogen at 200 ppm concentration. At the highest concentration (500 ppm) hexaconazole also completely inhibited the mycelial growth (100% mycelial growth inhibition) of the pathogen followed by difenoconazole and bitertenol which caused 74.30 and 66.70 per cent mean mycelial inhibition, respectively. Even at the highest concentration (500ppm), myclobutanil resulted in lowest mean mycelial growth inhibition of only61.50 per cent.

The *in-vitro* evaluation of bio-control agents revealed that Tv-4 and Tv-2 was most efficacious and resulted in 48.20 and 47.06 per cent mean mycelial inhibition followed by Tv-1 with 45.93 per cent mean inhibition of mycelial growth. *Trichoderma viride*-5 (Tv-5) proved least efficious and resulted in only 26.40 per cent mean inhibition of mycelial growth (Table 3).

Table.1 *In-vitro* evaluation of non-systemic fungicides against *Fusarium oxysporum* causing tuber rot of kalazeera

Fungicides	Per cent inhibition of radial mycelial growth at concentration (ppm)					
	50	**100**	**250**	**500**	**1000**	**Mean**
Captan 50WP	44.40 (41.77)	70.06 (56.84)	71.86 (57.98)	72.20 (58.20)	81.43 (64.48)	**67.99**
Mancozeb 75 WP	44.03 (41.03)	53.26 (46.87)	53.26 (46.87)	64.16 (53.23)	80.20 (63.62)	**58.98**
Copper oxychloride 50 WP	25.90 (30.59)	28.33 (32.11)	27.06 (31.26)	29.56 (32.93)	38.20 (38.17)	**29.81**
Antracol 70 WP	37.00 (37.46)	38.23 (38.23)	41.96 (40.37)	50.60 (45.34)	62.90 (52.51)	**46.14**
Dodine 65WP	61.66 (51.75)	75.33 (60.17)	81.43 (64.48)	92.96 (74.67)	100.00 (90.00)	**82.27**
Zineb	35.76 (36.76)	43.13 (41.05)	43.16 (41.06)	51.83 (46.04)	60.43 (51.02)	**46.86**
Mean	**41.45**	**51.18**	**53.12**	**60.22**	**70.52**	
CD (P=0.05) **Non-systemic fungicide = (2.1)** **Concentration = (1.9)** **Fungicide × Concentration = (4.70)**						

*Figures within parentheses are arc sign transformed values

Table.2 *In-vitro* evaluation of systemic fungicides against *Fusarium oxysporum* causing tuber rot of kalazeera

Fungicides	Per cent inhibition of radial mycelial growth at concentration (µg ml^{-1})					
	25	50	100	200	500	Mean
Hexaconozole 5EC	53.80 (41.17)	61.50 (51.64)	66.60 (54.69)	74.30 (59.53)	100.00 (90.00)	71.24
Myclobutanil 10 WP	43.53 (41.28)	51.23 (45.70)	51.20 (45.68)	56.33 (48.63)	61.50 (51.64)	52.76
Carbendazim 50WP	82.00 (64.89)	84.56 (66.86)	84.60 (66.89)	100.00 (90.00)	100.00 (90.00)	90.23
Difenconazole 25 EC	51.23 (45.70)	64.03 (53.14)	64.06 (53.16)	69.16 (56.26)	74.30 (59.53)	60.84
Bitertanol 25WP	48.66 (44.23)	56.33 (48.63)	64.10 (53.19)	66.63 (54.71)	66.70 (54.74)	64.56
Mean	55.84	63.53	66. 11	73.28	80.50	
CD (P=0.05) Fungicide = (1.27) Concentration = (125) Fungicide × Concentration = (2.33)						

Figures within parentheses are arc sign transformed values

Table.3 *In-vitro* evaluation of bio-control agents against *Fusarium oxysporum* causing tuber rot of Kala zeera

Bio-control agents	Per cent inhibition in radial mycelial growth of the pathogen
***Trichoderma viridi*-1 (Tv-1)**	45.93 (42.66)
***Trichoderma viridi*-2 (Tv-2)**	47.06 (43.31)
***Trichoderma viridi*-3 (Tv-3)**	37.70 (34.87)
***Trichoderma viridi*-4 (Tv-4)**	48.20 (43.96)
***Trichoderma viridi*-5 (Tv-5)**	26.40 (30.87)
***Trichoderma harzianum*-1 (Th-1)**	36.73 (37.30)
***Trichoderma harzianum* -2 (Th-2)**	39.60 (38.99)
CD (P=0.05) (2.01)	

Figures within parentheses are arc sign transformed values

Evaluation of fungicides and bio-control agents *in-vitro* is indispensible for determining their efficacies before they are finally take to field for evaluation. *In-vitro* evaluation helps in selecting the best fungicide(s) and bio-control agent(s) among the array of fungicides and bio-control agents. *In-vitro* evaluation also determines the best concentration at which the fungicide(s) is/are most effective. Evaluation of fungicides in field without *in-vitro* evaluation at different concentrations is practically not feasible and would include huge land, labour and costs. Kala zeera is a niche crop of Kashmir and not much work has been done on diseases of this important cash crop. Our results are in conformity with those reported by Aghnoom *et al*, (1999), Aghnoom *et al.*, (2002), Ghasolia and Jain (2004), Khan *et al.*, (2012). Generally, all the treatments checked the activities of the pathogen (*Fusarium oxysporum*) and consequently promote the growth of Kala zeera. In the present study, among biological control agents, *Trichoderma viridi* proved most effective and these findings were completely in agreement with Saikia *et al.*, 2003; Ghasolia and Jain, 2004 and Postma *et al*., 2003.

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How to cite this article:

Sabiya Bashir, Mohammad Najeeb Mughal, Seeratun Nissa, Zahoor A. Dar, S.A. Hakeem, Shabeena Majeed, Shaheena Nagoo and Sabeena Naseer. 2018. Efficacy of Some Fungicides and Bio-control Agents against Tuber Rot (*Fusarium oxysporum*) of Kalazeera (*Bunium persicum*). *Int.J.Curr.Microbiol.App.Sci.* 7(07): 3752-3757.
doi: https://doi.org/10.20546/ijcmas.2018.707.434