Article AR2460

DOI: HTTPS://DOI.ORG/10.23910/1.2021.2460

International Journal of Bio-resource and Stress Management

October 2021

Crossref

Print ISSN 0976-3988

Online ISSN 0976-4038

IJBSM 2021, 12(5):523-531

Research Article *Stress Management*

Efficacy of Fungicides against Soil Borne and Grapevine Pathogens under *In vitro* Conditions

Anjali Singh[1], Ratna U. Thosar[2*], Vijayshree Chavan[2] and Sujoy Saha[2]

[1]Amity Institute of Organic Agriculture, Amity University, Amity Rd, Sector 125, Noida, Uttar Pradesh (201 301), India
[2]ICAR - National Research Centre for Grapes, Pune, Manjiri Farm, P B, No 3, Solapur Rd, Pune, Maharashtra (412 307), India

Open Access

Corresponding Author

Ratna U. Thosar

e-mail: ratnathosar.1234@gmail.com

Citation: Singh et al., 2021. Efficacy of Fungicides against Soil Borne and Grapevine Pathogens under *In vitro* Conditions. International Journal of Bio-resource and Stress Management 2021, 12(5), 523-531. HTTPS://DOI.ORG/10.23910/1.2021.2460.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

Abstract

In vitro bio-efficacy of the novel copper (NC101 and NCP102) and phosphonate (PN103 and PMN104) based fungicides against various soil borne and grape vine pathogens was evaluated at ICAR-National Research Centre for Grapes, Pune, Maharashtra, India during February - April 2021. The fungicides were screened against five bacterial pathogens viz. *Xanthomonas campestris* pv. *citri, X. campestris* pv. *campestris, X. campestris* pv. *punicae, X. campestris* pv. *viticola* and *X. oryzae* pv. *oryzae* and 10 fungal pathogens viz. *Rhizoctonia bataticola, Sclerotium rolfsii, Cladosporium sp, Alternaria alternata, Colletotrichum gloeosporioides, Penicillium notatum, Magnaporthe oryzae, Fusarium oxysporium, Macrophomina phaseolina* (Soyabean isolate) and *Macrophomina phaseolina* (Jute isolate) at different concentrations. Results indicated that among all the tested fungicides viz. NC101, NCP102, PN103 and PMN104, phosphonate based fungicides (PN103 and PMN104) were highly effective against bacterial isolates with zone of inhibition ranging between 8.75 - 31.12 mm in which *X. campestris* pv. *viticola* was found to express least inhibition zone. In case of pathogenic fungal isolates, similar trend was observed, most of the isolates showed cent percent inhibition at higher concentration of PN103 and PMN104. However *Sclerotium rolfsii* showed least or no inhibition when tested at different concentrations of fungicides. The chemicals exhibited wide range of inhibition and it was found to increase steadily with increase in concentrations of the test fungicides.

Keywords: Bio-efficacy, phosphonates, copper, pathogens, inhibition, grapevine

1. Introduction

Grape (*Vitis vinifera* L.) is an important fruit crop of India having both commercial and nutritional value. It is consumed as fresh or as processed food like wine and raisins. Production of grapevines is threatened by biotic (viruses, bacteria, fungi and insects) and abiotic stresses (i.e. drought, winter cold) (Heidari et al., 2021). Grape has been affected by several diseases which mostly reduce the yield and deteriorate the fruit and wine quality. Downy mildew [*Plasmopara viticola* (Berk and Curtis) Berlese and De toni], Powdery mildew [*Uncinula necator* (Schw.) Burn], Anthracnose [*Gloeosporium ampelophagum* (Pass) Sacc. (Perfect stage: *Elsinoe ampelina* (DeB) Shear)] and Bacterial leaf spot [*Xanthomonas campestris* pv. *viticola* (Nayudu) Dye] are the major constraints in grapevine cultivation (Vinothini et al., 2014; Liu et al., 2018; Thiery et al., 2018; Li et al., 2019; Muthukumar et al., 2019; Rama et al., 2020; Kim et

Article History

RECEIVED on 25th June 2021 RECEIVED in revised form on 18th September 2021 ACCEPTED in final form on 26th October 2021

al., 2021). Application of fungicides is the most convenient and predominant way for disease control.

Soilborne diseases are considered a major limitation to crop production. Soilborne plant pathogens such as *Rhizoctonia* spp, *Fusarium* spp, *Alternaria* spp, *Sclerotium* spp and *Macrophomina* spp. can cause 50%–75% yield loss for many crops such as wheat, cotton, maize, vegetables, fruit and ornamentals as reported to date (Mihajlovic et al., 2017; Baysal-Gurel et al., 2018). The use of fungicides against soilborne plant pathogens can also help to manage some diseases. The efficacy of fungicides is influenced by many biological and environmental factors that directly influence the metabolic activities of fungal cells (Panek et al., 2014; Koziróg et al., 2016; Kukowski et al., 2017; Teaca et al., 2019; Bremer et al., 2021). Sometimes critical concentrations are not effective in long-term, as pathogens can become resistant to the fungicide (Lal et al., 2015; Halleen et al., 2017; Kuai et al., 2017; Diaz et al., 2020; Vielba-Fernandez et al., 2020). Frequent and indiscriminate use can increase environmental and health concerns and lead to development of fungicide resistance (Christopher et al., 2010). Plant fungicides based on synthetic chemicals are extensively used in agriculture. In fact, there are now more than 113 active ingredients registered as fungicides worldwide (Makovitzki et al., 2007). However the ineffectiveness of some fungicides in the field not only caused a monetary loss but may also have deleterious effects on crops and environment. Phosphonates have been used extensively as crop protectants in horticulture since the late 1970s. They are the most versatile, safe, and potential fungicides against soilborne diseases having unique in their ability to reduce the disease (Dann and McLeod, 2021). Phosphonates fungicides control disease by direct action as well as indirect action as a systemic acquired resistance initiator. They are very effective against oomycete diseases including *Phytophthora* aerial blight and downy mildew. They also work very well at times on *Pythium* root rot and powdery mildew as well as some bacterial leaf spots (Cook et al., 2009). However, Copper as fungicide acts as a broadspectrum biocide at higher concentrations due to its interaction with nucleic acids, disruption of enzyme active sites, interference with the energy transport system, and finally the disruption of the integrity of cell membranes (Lamichhane et al., 2018).

In vitro evaluation of fungicides offers useful information regarding efficacy against pathogens. It could be carried out in a short time and provide guidelines for future field testing. Therefore, the present study was conducted to assess the *in vitro* bioefficacy of four novel fungicides namely NC101, NCP102, PN103 and PMN104 with different isolates of soil borne and grapevine pathogens.

2. Materials and Methods

2.1. Fungicide

Four novel fungicides considered for the study were; NC101 (Contains modified copper particles), NCP102 (NC101 impregnated with phytohormones), PN103 (phosphonates with mixture of macro and micro-nutrients), PMN104 (PN103 with additional micro nutrients). *In vitro* bioefficacy of four novel fungicides; NC101 (500, 1000, 2000, 3000 and 4000 ppm), NCP102 (250, 500, 1000, 2000 and 3000 ppm), PN103 (500, 1000, 2000, 3000 and 4000 ppm) and PMN104 (500, 1000, 2000, 3000 and 4000 ppm) were evaluated.

2.2. Pathogens

Five bacterial pathogen viz. *Xanthomonas campestris* pv. *citri* (Citrus canker), *Xanthomonas campestris* pv. *campestris* (Black rot of cabbage), *Xanthomonas campestris* pv. *punicae* (Bacterial leaf blight of pomegranate), *Xanthomonas campestris* pv. *viticola* (Bacterial leaf spot of grapes) and *Xanthomonas oryzae* pv. *oryzae* (Bacterial leaf blight of rice) and 10 fungal pathogens viz. *Rhizoctonia bataticola* (Damping off of chillies), *Sclerotium rolfsii* (Stem rot of paddy), *Cladosporium sp* (Bunch rot in grapes), *Alternaria alternata* (Early blight of brinjal), *Colletotrichum gloeosporioides* (Anthracnose of grapes), *Penicillium notatum* (Blue mold of citrus), *Magnaporthe oryzae* (Blast of paddy), *Fusarium oxysporium* (Wilt of tomato), *Macrophomina phaseolina* (Soyabean isolate) (Charcoal rot of soyabean) and *Macrophomina phaseolina* (Jute isolate) (Stem rot of jute) were used in the study. All the pathogens were procured from culture collection of ICAR- National Research Centre for Grapes. Fungal strains were cultured and preserved on Potato Dextrose Agar (Hi Media MH096) and bacterial cultures were maintained on Nutrient Agar (Hi Media MM012).

2.3. Bio-efficacy of fungicides against Xanthomonas species (Paper Disc method)

The bioefficacy of novel fungicides against bacterial pathogens were studied using paper disc method (Meena et al., 2004). The derived concentrations of the novel fungicides were freshly prepared in sterile distilled water and the bacterium was multiplied by inoculating the loopful culture in 150 ml conical flask containing 50 ml of nutrient broth medium. The inoculated flasks were incubated at 28±1°C for 48 h.

The bacterial culture in broth was seeded to lukewarm Nutrient agar. The seeded medium (15–20 ml) was poured in sterilized Petriplates of diameter 90 mm and allowed to solidify. The sterilized filter paper (Whatman filter paper no. 1) discs (5 mm in diameter) were soaked in the solutions of different concentrations of above mentioned chemicals for 10 minutes. The chemical impregnated discs were placed onto the lawn with the help of sterilized forceps. Then the plates were incubated at 28±1°C for 72 hr. Lysis of the bacterial lawn around the disc were recorded and the results of sensitivity were reported as the zone of inhibition. The sterilized paper discs impregnated with sterile distilled water served as control.

2.4. Bio-efficacy of fungicides against fungal pathogens (Poison food technique)

For evaluation of different fungicides, "Poisoned Food Technique" developed by Nene and Thapliyal (1979) was followed. Mother culture was prepared in Potato Dextrose

Agar (PDA) media from the pure culture which was used as the mycelium source of fungus. Different concentrations of each novel fungicide were added to 200 ml of PDA medium for obtaining various concentrations of fungicidal suspension. 15 ml of poisoned medium was poured in each Petri plate and allowed to solidify. Negative checks i.e. control plates were maintained without addition of the fungicides to the media. Six mm diameter piece of fungal mycelium was taken from seven-day old cultured plate and kept at the center of the poisoned plates. The treated plates were incubated at 25±2°C. Observations on radial mycelial growth colony diameter were recorded at the time when untreated control plates were fully covered with mycelial growth of the test pathogen. Percent inhibition of fungal growth was calculated using the following formula (Vincent, 1947):

Percent growth inhibition = A-B/A *X* 100

Where,

A=Colony growth of the fungus in control plate

B=Colony growth of the fungus in treated plate

2.5. Statistical analysis

Recorded data were analyzed in Completely Randomised Design (CRD) through Analysis of Variance (ANOVA) and treatments means were compared by Fisher's Least Significant Difference (LSD) test. Data was processed statistically through SAS (9.3) software.

3. Results and Discussion

3.1. In vitro evaluation of novel fungicides against different Xanthomonas species

In vitro efficacy of novel fungicides at five different concentrations were evaluated by measuring the diameter of the inhibition zone of different *Xanthomonas* species 72 hours after inoculation. *Xanthomonas campestris* pv. *citri* showed highest zone of inhibition of 19.25 mm against fungicide PMN104 at 500 ppm but at 4000 ppm fungicide PN103 displayed maximum inhibition zone of 27.50 mm. Least zone of inhibition was expressed against fungicide NC101 that is 16 mm at 500 ppm and 23.75 mm at 4000 ppm. For *X. oryzae* pv. *oryzae,* fungicide NC101 displayed least inhibition zone of 14.50 mm at 500 ppm and 26.00 mm at 4000 ppm as compared to other fungicides. Phosphonate formulation PMN104 showed highest inhibition at all concentrations. Against pathogenic bacteria *Xanthomonas campestris* pv. *punicae* both the phosphonate formulations showed better inhibitory results. PMN104 expressed highest zone of inhibition of 16 mm at 500 ppm and 25.75 mm at 4000 ppm. However, fungicide NCP102 displayed smallest inhibition zone of 12.12 mm at 250 ppm and 19.12 mm at 3000 ppm. Fungicide PN103 at 500 ppm displayed best inhibitory results against *Xanthomonas campestris* pv. *campestris* whereas at 4000 ppm PMN104 showed better inhibition. Fungicide NCP102 showed least inhibition zone at all concentrations. *X.campestris* pv. *viticola* with fungicides NCP102 and NC101 showed minimum inhibition zone of 6.50 mm at 500 ppm and 12.75 mm at 4000 ppm respectively. Highest zone of inhibition was displayed by fungicide PMN104 at all concentrations.

Kumar et al., 2018 and Mondal et al., 2011 reported the bio-efficacy of copper based fungicides against *Xanthomonas axonopodis* pv. *punicae*. The above results are also in confirmation with the experiment conducted by Heydarpanah et al. in 2019 where he studied the efficacy of different copper compounds against 30 isolates of *Xanthomonas citri*. Several commercial bactericides aim to treat diseases caused by *Xanthomonas* sp and major issue associated with these control measures is the decrease in plant immunity against further attack and other pathogens. Several laboratory studies showed that application of phosphite compounds improves plant defence (Burra et al., 2014; Eshraghi et al., 2011; Lim et al., 2013). The present study screened the efficacy of novel phosphonate fungicides PN103 and PMN104 against different *Xanthomonas* species. Chase et al., 1993 and Moragrega et al., 1998 also reported the efficacy of phosphonate for control of some bacterial diseases (Table 1).

Table 1: Inhibition zone in mm of various *Xanthomonas* sp. against different novel fungicides

Treatment	NC101					NCP102				
Concentration	500	1000	2000	3000	4000	250	500	1000	2000	3000
Xanthomonas campestris pv. citri	16.00^{b}	16.62^{bc}	18.00^{bcd}	19.25^{efgd}	23.75^{jk}	17.75^{bcd}	19.00^{efd}	20.25^{efgh}	21.00^{ifgh}	25.50^{mlk}
Xanthomonas oryzae pv. oryzae	14.50^{b}	16.62^{d}	20.00^{fg}	23.87^{j}	26.00^{k}	17.25^{d}	20.37^{g}	21.50^{h}	23.75^{j}	27.25^{l}
Xanthomonas campestris pv. punicae	12.25^{b}	15.12^{d}	17.62^{f}	18.25^{gf}	21.62^{i}	12.12^{b}	14.00^{c}	15.00^{d}	17.87^{f}	19.12^{h}
Xanthomonas campestris pv. campestris	16.12^{d}	19.50^{gih}	20.37^{jih}	24.25^{l}	26.75^{m}	10.75^{b}	14.62^{c}	16.11^{d}	18.12^{ef}	20.75^{ji}
Xanthomonas campestris pv. viticola	6.75^{b}	8.25^{cd}	10.75^{e}	11.75^{ef}	12.75^{f}	6.50^{b}	7.50^{cb}	9.25^{d}	15.12^{g}	16.87^{h}

Table 1: Continue...

Treatment	PN103					PMN104					LSD
Concentration	500	1000	2000	3000	4000	500	1000	2000	3000	4000	
Xanthomonas campestris pv. citri	18.25^{ecd}	22.75^{ij}	23.75^{jk}	26.00^{lm}	27.50^{m}	19.25^{efgd}	21.25^{igh}	22.25^{ijh}	23.50^{jk}	25.25^{lk}	2.19
Xanthomonas oryzae pv. oryzae	16.50^{d}	19.12^{fe}	22.50^{i}	23.37^{ij}	27.00^{l}	15.50^{c}	19.00^{e}	23.75^{j}	27.37^{l}	30.00^{m}	0.98
Xanthomonas campestris pv. punicae	15.25^{d}	18.75^{gh}	23.00^{j}	24.75^{k}	26.75^{m}	16.00^{e}	18.75^{gh}	23.23^{j}	24.25^{k}	25.75^{l}	0.65
Xanthomonas campestris pv. campestris	17.37^{ed}	18.75^{gf}	21.25^{jk}	27.37^{m}	30.75^{n}	16.25^{d}	19.25^{gfh}	22.50^{k}	26.37^{m}	31.12^{n}	0.66
Xanthomonas campestris pv. viticola	8.75^{cd}	12.75^{f}	19.25^{i}	21.50^{j}	22.37^{j}	10.75^{e}	14.75^{g}	21.12^{j}	24.00^{k}	26.75^{l}	0.87

*: Figure in parenthesis indicates the angular transformed values; **: Means with the same letter are significantly different; ***: Inhibition zone of untreated control was 0.00 mm for each bacterial species

3.2. In vitro evaluation of novel fungicides against different fungal pathogens

In vitro evaluation of novel fungicides against different fungal pathogens were carried out by following the "Poison food technique". The results are presented in Table 2. The best inhibition of the fungal growth was exhibited by the phosphonate fungicides as compared to copper based fungicides. In case of *Rhizoctonia bataticola* all treatments except NCP101 at 500 ppm concentration were capable of reducing the fungal growth as compared to untreated control. Among all fungicides, maximum inhibition was observed with fungicide PMN104 that is 13.06% inhibition at 500 ppm and 100 % inhibition at 2000 ppm, 3000 ppm and 4000 ppm PN103 expressed inhibition of 6.39% at 500 ppm and 100%

Table 2: Efficacy of different novel fungicides against pathogenic fungi

Treatment	NC101					NCP102				
Conc in ppm	500	1000	2000	3000	4000	250	500	1000	2000	3000
Rhizoctonia bataticola	0.00 (0.00)^{a}	5.56 (13.59)^{b}	8.61 (16.99)^{c}	29.72 (33.02)^{f}	100 (90.00)^{j}	0.00 (0.00)^{a}	5.56 (13.59)^{b}	8.61 (16.99)^{c}	23.61 (29.03)^{e}	35.28 (36.42)^{g}
Sclerotium rolfsii	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	1.11 (4.28)^{b}	1.67 (6.42)^{bc}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	8.89 (17.19)^{e}	15.00 (22.72)^{f}	25.28 (30.16)^{h}
Cladosporium sp	7.94 (16.20^{b}	12.06 (20.29)^{c}	18.24 (25.26)^{e}	30.88 (33.75)^{g}	38.82 (38.53)^{h}	14.71 (22.49)^{d}	19.12 (25.92)^{e}	25.88 (30.57)^{g}	31.18 (33.94)^{g}	37.94 (38.01)^{h}
Alternaria alternata	6.39 (14.60)^{b}	25.56 (30.34)^{d}	46.39 (42.92)^{fg}	52.78 (46.59)^{h}	69.72 (56.63)^{k}	7.50 (15.73)^{bc}	44.44 (41.81)^{f}	49.17 (44.52)^{g}	56.67 (48.83)^{i}	62.50 (52.24)^{j}
Colletotrichum gloeosporioides	0.00 (0.00)^{a}	24.17 (29.42)^{c}	40.56 (39.55)^{e}	54.44 (47.55)^{f}	60.00 (50.77)^{g}	0.00 (0.00)^{a}	42.22 (40.51)^{e}	51.94 (46.11)^{f}	60.28 (50.95)^{g}	61.67 (51.74)^{g}
Penicillium notatum	8.33 (16.55)^{c}	11.11 (19.47)^{d}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	5.56 (13.63)^{b}	64.17 (53.24)^{f}	71.11 (57.49)^{g}	76.39 (60.95)^{h}	81.11 (64.28)^{i}
Magnaporthe oryzae	3.33 (8.95)^{b}	6.39 (14.60)^{c}	17.22 (24.50)^{de}	25.56 (30.34)^{f}	36.67 (37.25)^{g}	1.94 (5.55)^{b}	8.89 (17.19)^{c}	13.06 (21.11)^{d}	18.89 (25.72)^{e}	29.72 (33.02)^{f}
Fusarium oxysporium	6.94 (15.09)^{b}	15.00 (22.72)^{c}	22.22 (28.13)^{d}	56.94 (48.99)^{j}	63.33 (52.74)^{k}	20.28 (26.73)^{d}	36.67 (37.25)^{f}	46.39 (42.92)^{h}	40.83 (39.71)^{g}	53.06 (46.75)^{i}
Macrophomina phaseolina (Soyabean isolate)	0.00 (0.00)^{a}	5.56 (13.60)^{b}	15.28 (22.95)^{c}	29.17 (32.67)^{d}	31.94 (34.37)^{d}	6.11 (14.30)^{b}	64.17 (53.22)^{f}	69.44 (56.45)^{g}	70.83 (57.32)^{hg}	75.00 (60.05)^{ji}
Macrophomina phaseolina (Jute isolate)	5.56 (13.63)^{b}	14.17 (22.05)^{c}	26.94 (31.26)^{f}	31.94 (34.40)^{g}	53.33 (46.91)^{i}	24.17 (29.42)^{e}	43.61 (41.32)^{h}	100.00 (90.00)^{k}	100.00 (90.00)^{k}	100.00 (90.00)^{k}

Table 2: Continue...

Treatment	PN103					PMN104					LSD
Conc in ppm	500	1000	2000	3000	4000	500	1000	2000	3000	4000	
Rhizoctonia bataticola	6.39 (14.60)^{b}	34.72 (36.08)^{g}	74.72 (59.83)^{i}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	13.06 (21.11)^{d}	63.61 (52.90)^{h}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	1.82
Sclerotium rolfsii	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	5.28 (13.26)^{d}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	3.06 (8.59)^{c}	20g (26.52)^{g}	3.08
Cladosporium sp	14.71 (22.49)^{d}	42.35 (40.60)^{i}	72.65 (58.49)^{k}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	18.24 (25.26)^{e}	52.35 (46.35)^{j}	72.65 (58.49)^{k}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	1.83
Alternaria alternata	7.50 (15.73)^{bc}	31.11 (33.88)^{e}	100.00 (90.00)^{l}	100.00 (90.00)^{l}	100.00 (90.00)^{l}	8.33 (16.66)^{c}	68.61 (55.94)^{k}	100.00 (90.00)^{l}	100.00 (90.00)^{l}	100.00 (90.00)^{l}	1.98
Colletotrichum gloeosporioides	6.94 (15.09)^{b}	64.72 (53.57)^{h}	100.00 (90.00)^{i}	100.00 (90.00)^{i}	100.00 (90.00)^{i}	36.67 (37.25)^{d}	67.22 (55.08)^{h}	100.00 (90.00)^{i}	100.00 (90.00)^{i}	100.00 (90.00)^{i}	1.81
Penicillium notatum	20.00 (26.52)^{e}	75.00 (60.03)^{h}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	64.72 (53.57)^{f}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	1.56
Magnaporthe oryzae	65.28 (53.90)^{h}	73.33 (58.93)^{i}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	62.78 (52.41)^{h}	73.89 (59.29)^{i}	100 (90.00)^{j}	100 (90.00)^{j}	100 (90.00)^{j}	3.44
Fusarium oxysporium	29.72 (33.02)^{e}	62.50 (52.25)^{k}	100.00 (90.00)^{l}	100.00 (90.00)^{l}	100.00 (90.00)^{l}	41.11 (39.87)^{g}	53.61 (47.07)^{ij}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	1.99
Macrophomina phaseolina (Soyabean isolate)	5.56 (13.60)^{b}	52.22 (46.27)^{e}	74.17 (59.50)^{hi}	86.94 (68.75)^{l}	100.00 (90.00)^{m}	5.56 (13.60)^{b}	16.67 (21.10)^{c}	77.78 (61.90)^{jk}	79.72 (63.25)^{k}	100.00 (90.00)^{j}	2.23
Macrophomina phaseolina (Jute isolate)	20.83 (27.12)^{d}	43.06 (41.00)^{h}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	14.44 (22.26)^{c}	65.28 (53.91)^{j}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	100.00 (90.00)^{j}	1.56

*: Figure in parenthesis indicates the angular transformed values; **: Means with the same letter are not significantly different; ***: % inhibition of untreated control was 0.00 for all pathogenic fungi

inhibition at 3000 ppm and 4000 ppm both. The present study confirmed to the reports of Rajendraprasad et al., 2017 and Kumar et al., 2017 who reported the inhibition of *R. solani* by copper oxy chloride 50% WP. However copper based fungicide NC101 displayed no inhibition of *R. bataticola* at minimum concentration i.e. 500 ppm, but cent percent (100%) inhibition at maximum concentration of 4000 ppm was observed. At lowest concentration NCP102 also did not showed any inhibition but increased to 35.28% inhibition compared to untreated control at 3000 ppm. However the above results were in contradiction with the previous findings of Amrutha et al., 2019 where copper hydroxide and copper oxychloride displayed better inhibition of *R. solani* than potassium phosphonate. The efficacy tests undertaken against *Sclerotium rolfsii* revealed that, among the novel fungicides taken into account, none of the fungicide significantly inhibited the pathogen except nominal inhibition at higher concentrations. Fungicide NC101 did not inhibit the pathogenic fungi in any of the concentrations. *Sclerotium rolfsii* was relatively merely inhibited at 2000 ppm (15% inhibition) and (25.28%) 3000 ppm by NCP102. None of the phosphonates exhibited high degree of inhibition against *Sclerotium rolfsii* and only 5.28% and 20% inhibition was observed at 4000 ppm by PN103 and PMN104 respectively. The results were in conformity with the findings of Johnson et al., 2000 and Shirsole et al., 2019 who reported that Copper-oxy-chloride was not found effective in inhibiting the growth and sclerotia production of *Sclerotium rolfsi*.

In case of *Cladosporium cladosporioides* the phosphonate PN103 showed 14.71% inhibition at lowest concentration i.e. 500 ppm. At 3000 ppm and at 4000 ppm the fungicide PN103 completely inhibited the fungi. Same were the results with PMN104 where complete inhibition of fungi was seen at maximum concentration of 3000 ppm and 4000 ppm. In case of copper based fungicides NC101 exhibited 7.94% inhibition at 500 ppm which intensified upto 38.82% at 4000 ppm against *Cladosporium* sp. The fungicide NCP102 displayed 14.71% and 37.94% inhibition at 250 ppm and 3000 ppm respectively. Bioefficacy of novel fungicides against *Alternaria alternata* exhibited that among all the fungicide tested phosphonate PMN104 showed the highest inhibition of the fungi followed by another phosphonate PN103. PN103 and PMN104 showed no growth which means 100% inhibition at 2000 ppm, 3000 ppm and 4000 ppm. Similarly Yogev et al., 2006 reported that Potassium phosphite arrested mycelial growth of *A. solani* completely. Balai et al., 2018 and Kaur et al., 2020 reported significant inhibition of *Alternaria* sp. by Copper

oxychloride at various concentrations. The present study was in cognizance with the earlier studies conducted the fungicide NC101 displayed 6.39% inhibition at 500 ppm and 69.72% at 4000 ppm. *Alternaria alternata* when tested against fungicide NCP102 showed inhibition of 7.50% at lowest concentration (250 ppm) which increased to 62.50% at highest concentration (3000 ppm).

The efficacy of novel fungicides tested against *Colletotrichum gloeosporioides* showed that the phosphonates (PPl01 and PMN104) displayed greater inhibition than copper based fungicides (NC101 and NCP102). The fungus when tested against NC101 showed no inhibition at 500 ppm but increased upto 60% inhibition at 4000 ppm and was the same case with NCP102. In case of PN103 and PMN104 the inhibition increased from 6.94% and 36.67% respectively at 500 ppm to 100 % inhibition at 2000 ppm, 3000 ppm and 4000 ppm. The results are in cognizance with the findings of Rampersad et al., 2012 who reported that Fosetyl-aluminum was found to be effective against *Colletotrichum* spp. However in case of copper fungicides minor contradiction was observed with the recent findings of Ntasiou et al., 2021 who reported better inhibition of *Colletotrichum* spp by novel copper nanoparticles.

Roberto et al., 2019 reported fungicidal activity of copper nanoparticles (CuNPs) against *Penicillium digitatum* where complete growth inhibition was recorded at 20 ug ml^{-1} for *P. digitatum*. In the present study all the fungicides tested significantly inhibited the pathogenic fungus. Fungicide PMN104 highly inhibited the fungal mycelium than other novel fungicides followed by another phosphonate PN103. The fungicide NC101 totally inhibited the fungi i.e. 100% inhibition at 2000 ppm, 3000 ppm and 4000 ppm and NCP102 exhibited 5.56% inhibition at 250 ppm which escalated to 81.11% at 3000 ppm. The fungicide PN103 and PMN104 showed cent percent inhibition (100%) at 2000 ppm, 3000 ppm and 4000 ppm. Amiri and Bompeix (2011) also reported complete inhibition of mycelial growth and conidial germination of *Penicillium expansum* by potassium phosphite.

The bio-efficacy test of different novel chemicals against *Magnaporthe oryzae* revealed that the fungicide NC101 least inhibited the fungus and the fungicide PN103 maximally inhibited the fungal mycelium. NC101 exhibited 3.33% inhibition at lowest concentration i.e. 500 ppm and 36.67% inhibition at highest concentration i.e. 4000 ppm. NCP102 displayed 1.94% and 29.72% inhibition at 250 ppm and 3000 ppm respectively. *Magnaporthe oryzae* when tested against phosphonates PN103 and PMN104 showed 100% inhibition at 2000 ppm, 3000 ppm and 4000 ppm respectively. Similarly Hajano et al., 2012 reported better inhibition of *Magnaporthe oryzae* by Fosetyl-aluminium than copper oxychloride. *Fusarium oxysporum* manifested that the phosphonates (PPl01 and PMN104) had higher inhibition than copper based fungicides (NC101 and NCP102). Kanhed et al., 2014 reported that copper nanoparticles demonstrated significant antifungal activity against plant pathogenic fungi *Fusarium oxysporum*.

In the present study the fungi when tested against NC101 showed 6.94% inhibition at 500 ppm which increased upto 63.33% inhibition at 4000 ppm. Similarly NCP102 exhibited 20.28% inhibition at 250 ppm but at 3000 ppm 53.06% inhibition was displayed. The Phosphonate PN103 and PMN104 at initial concentration that is 500 ppm exhibited inhibition of 29.72% and 41.11% respectively at 500 ppm (@ 62.50% and 53.61%) cent percent inhibition was observed at 2000 ppm, 3000 ppm and 4000 ppm. The results of present investigation have resembled with earlier records of Maitlo et al., 2014 who reported efficacy of Fosetyl AL in complete inhibition of mycelial growth of *F. oxysporum.* Iqbal et al*.,* 2020 evaluated Copper oxychloride at a concentration of 50 ppm gave the minimum inhibition (12.50%). In the current study both the isolates of *Macrophomina phaseolina* showed better inhibition with the phosphonates (PN103 and PMN104) than the copper based fungicides (NC101 and NCP102) (Figure 1).

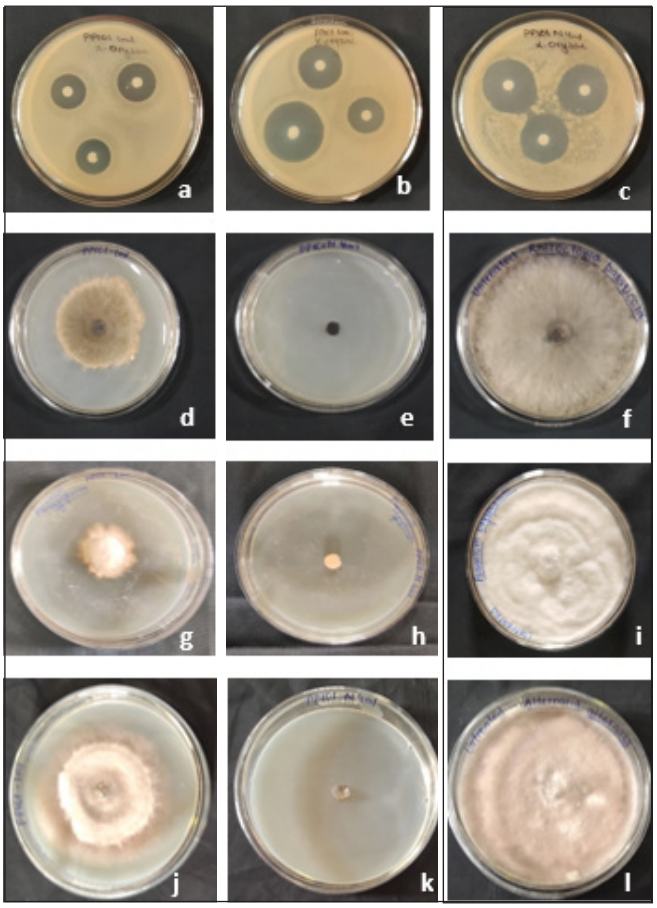

Figure 1: Zone of inhibition in *Xanthomonas oryzae* pv. *oryzae* by (a) PN103 1000 ppm (b) PN103 4000 ppm (c) PMN104 4000 ppm. Radial growth of *Rhizoctonia bataticola* (d) PN103 1000 ppm (e) PMN104 4000 ppm (f) Untreated control. Radial growth of *Fusarium oxysporium* (g) PN103 1000 ppm (h) PMN104 4000 ppm (i) Untreated control. Radial growth of *Alternaria alternata* (g) PN103 1000 ppm (h) PMN104 4000 ppm (i) Untreated control.

Results indicated that the fungicides tested at various concentrations exhibited a wide range of inhibition to various fungal pathogens over untreated control and it was found to increase steadily with increase in concentrations of the test fungicides.

4. Conclusion

The novel copper fungicides were least effective against *X. campestris* pv. *viticola* than other *Xanthomonas* sp. Similar trend was observed in case of phosphonates. At various concentrations, fungicides exhibited a wide range of inhibition zone in *Xanthomonas* spp, over untreated control and increase steadily with increase in concentrations. The phosphonates PN103 and PMN104 performed better than copper fungicides NC101 and NCP102 against studied pathogenic fungi. Field studies will be conclusive regarding the efficacy of the novel chemicals in controlling the pathogens and diseases.

5. References

Amiri, A., Bompeix, G., 2010. Control of *Penicillium expansum* with potassium phosphite and heat treatment. Crop Protection, 222–227.

Amrutha, P., Vijayaraghavan, R., 2019. Characterization of *Rhizoctonia solani* causing fruit rot of strawberry (*Fragaria×ananassa* Duch.) in wayanad and in vitro evaluation of fungicides, organic preparations and bioagents for its management. Journal of Horticultural Sciences 14, 155–160.

Balai, L., Singh, R., Sinha, A., Yadav, S., 2018. Evaluation of different fungicides and antagonists In vitro and In vivo condition against *Alternaria* blight of pigeonpea. Legume Research 43, 268–275.

Baysal-Gurel, F., Kabir, N., 2018. Comparative performance of fungicides and biocontrol products in suppression of *Rhizoctonia* root rot in viburnum. Journal of Plant Pathology & Microbiology 9, 451.

Bremer, M., Oktaee, J., Einer, D., Fischer, S., Wagenfuhr, A., 2021. Investigation on the potential of poplar bark from short-rotation coppices as bio-based fungicidal additives. Bioenergy Research, 1–10.

Burra, D., Berkowitz, O., Hedley, P.E., Morris, J., Resjeo, S., Levander, F., Liljeroth, E., Andreasson, E., Alexandersson, E., 2014. Phosphite-induced changes of the transcriptome and secretome in Solanum tuberosum leading to resistance against *Phytophthora infestans*. BMC Plant Biology, 14.

Chase, A.R., 1993. Efficacy of fosetyl-Al for control of some bacterial diseases on ornamentals. Plant Disease 77, 771–777.

Christopher, D.J., Raj, T.S., Rani, S.U., Udhayakumar, R., 2010. Role of defense enzymes activity in tomato as induced by *Trichoderma virens* against *Fusarium* wilt caused by *Fusarium oxysporum* f.sp. *lycopersici*. Journal of Biopesticides 3, 158–162.

Cook, P.J., Landschoot, P., Schlossberg, M., 2009. Inhibition of *Pythium* spp. and Suppression of *Pythium* blight of turfgrasses with phosphonate fungicides. Plant Disease 93, 809–814.

Dann, E., McLeod, A., 2021. Phosphonic acid: a long-standing and versatile crop protectant. Pest Manag Sci. 77(5), 2197–2208. Doi: 10.1002/ps.6156. Epub 2020 Nov 8. PMID: 33099862.

Diaz, M.A., Pereyra, M.M., Picon-Montenegro, E., Meinhardt, F., Dib, J., 2020. Killer yeasts for the biological control of postharvest fungal crop diseases. Microorganisms, 8.

Eshraghi, L., Anderson, J., Aryamanesh, N., Shearer, B., McComb, J., Hardy, G.E.S., O'Brien, P.A., 2011. Phosphite primed defence responses and enhanced expression of defence genes in *Arabidopsis thaliana* infected with *Phytophthora cinnamomi*. Plant Pathology, 60.

Hajano, J., Mubeen Lodhi, A., Mumtaz, A., Pathan, M., Ali, K., Serwar Shah, G., 2012. *In vitro* efficacy of fungicides, plant extracts and bio-control agents against Rice Blast pathogen *Magnaporthe oryzae*. Pakistan Journal of Botany, 1775–1778.

Halleen, F., Holz, G., 2017. An overview of the biology, epidemiology and control of *Uncinula necator* (powdery mildew) on grapevine, with reference to South Africa. South African Journal of Enology and Viticulture 22, 111–121.

Heidari, M.D., Turner, I., Ardestani-Jaafari, A., Pelletier, N., 2021. Operations research for environmental assessment of crop-livestock production systems. Agricultural Systems 193, 103208.

Heydarpanah, S., Rezaei, R., Taghavi, S., Charehgani, H., 2020. Efficacy of different copper compounds in the control of *Xanthomonas citri* subsp. *citri* pathotypes A and A. Journal of Phytopathology 168, 73–80.

Iqbal, U., Mukhtar, T., 2020. Inhibitory Effects of some Fungicides against *Macrophomina phaseolina* Causing Charcoal Rot. Pakistan Journal of Zoology, 52.

Johnson, M., Subramanyam, K.., 2000. *In-vitro* efficacy of fungicides against stem rot pathogen (*Sclerotium rolfsii*) of groundnut. Annals of Plant Protection Sciences 8, 255–257.

Kanhed, P., Birla, S., Gaikwad, S., Gade, A., Seabra, A., Rubilar, O., Duran, N., Rai, M., 2014. *In vitro* antifungal efficacy of copper nanoparticles against selected crop pathogenic fungi. Materials Letters 115, 13–17.

Kaur, M., Thind, S., Arora, A., 2020. Prevalence of ber black fruit spot (*Alternaria alternata*) and its management. Indian Phytopathology 73, 245–251.

Kim, J.S., Hassan, O., Chang, T., 2021. First report of *Colletotrichum aenigma* causing anthracnose of grape in Korea. Plant Disease. Available on: https://doi.org/10.1094/PDIS-11-20-2458-PDN

Kozirog, A., Rajkowska, K., Otlewska, A., Piotrowska, M., Kunicka-Styczynska, A., Brycki, B., Nowicka-Krawczyk, P., Koscielniak, M., Gutarowska, B., 2016. Protection of

historical wood against microbial degradation-selection and application of microbiocides. International Journal of Molecular Sciences, 17.

Kuai, X., Barraco, C., Despres, C., 2017. Combining fungicides and prospective NPR1-Based "Just-in-Time" immunomodulating chemistries for crop protection. Frontiers in Plant Science, 8.

Kukowski, K., Martinska, V., Sedgeman, C.A., Kuplic, P., Kozliak, E., Fisher, S., Kubatova, A., 2017. Fate of triazoles in softwood upon environmental exposure. Chemosphere 184, 261–268.

Kumar, A., 2018. Studies on *Xanthomonas axonopodis* pv. *punicae*, causing bacterial blight of pomegranate in Punjab. International Journal of Agriculture, Environment and Biotechnology, 11.

Kumar, V., Chaudhary, V., Kumar, D., Kumar, A., Sagar, S., Chaudhary, S., 2017. Efficacy of botanicals and fungicides against *Rhizoctonia solani* inciting sheath blight disease on Rice (*Oryza sativa* L.). Journal of Applied and Natural Science 9, 1916–1920.

Lal, M., Yadav, S., Chand, S., Kaushik, S.K., Singh, B., Sharma, S., 2015. Evaluation of fungicides against late blight (*Phytophthora infestans*) on susceptible and moderately resistant potato cultivars. Indian phytopathology 68, 345–347.

Lamichhane, J., Osdaghi, E., Behlau, F., Kohl, J., Jones, J.B., Aubertot, J., 2018. Thirteen decades of antimicrobial copper compounds applied in agriculture. A review. Agronomy for Sustainable Development 38, 1–18.

Li, Y., Heloir, M., Zhang, X., Geissler, M., Trouvelot, S., Jacquens, L., Henkel, M., Su, X., Fang, X., Wang, Q., Adrian, M., 2019. Surfactin and fengycin contribute to the protection of a *Bacillus subtilis* strain against grape downy mildew by both direct effect and defence stimulation. Molecular Plant Pathology 20, 1037–1050.

Lim, S., Borza, T., Peters, R.D., Coffin, R.H., Al-Mughrabi, K.I., Pinto, D.M., Wang-Pruski, G., 2013. Proteomics analysis suggests broad functional changes in potato leaves triggered by phosphites and a complex indirect mode of action against *Phytophthora infestans*. Journal of Proteomics, 93.

Liu, Y., Lan, X., Song, S., Yin, L., Dry, I., Qu, J., Xiang, J., Lu, J., 2018. In planta functional analysis and subcellular localization of the oomycete pathogen *Plasmopara viticola* candidate RXLR effector repertoire. Frontiers in Plant Science, 9.

Maitlo, S.A., Syed, R.N., Rustamani, M.A., Khuhro, R.D., Lodhi, A.M., 2014. Comparative efficacy of different fungicides against *Fusarium* wilt of Chickpea (*Cicer arietinum L.*). Pakistan Journal of Botany, 2305–2312.

Makovitzki, A., Viterbo, A., Brotman, Y., Chet, I., Shai, Y., 2007. Inhibition of fungal and bacterial plant pathogens *in vitro* and in planta with ultrashort cationic lipopeptides. Applied and Environmental Microbiology 73(20), 6629–6636.

Meena, C., Gopalakrishnan, J., 2004. Efficacy of Plant Extracts Against Bacterial Blight (*Xanthomonas oryzae* pv. *oryzae*) of Rice. Annals of Plant Protection Sciences 12, 344–346.

Mihajlovic, M., Rekanovic, E., Hrustic, J., Tanovic, B., 2017. Methods for management of soilborne plant pathogens. Pestic. Fitomedicina 32, 9–24.

Mondal, K., Mani, C., 2011. Investigation of the antibacterial properties of nanocopper against *Xanthomonas axonopodis* pv. *punicae*, the incitant of pomegranate bacterial blight. Annals of Microbiology 62, 889–893.

Moragrega, C., Manceau, C., Montesinos, E., 1998. Evaluation of drench treatments with phosphonate derivatives against *Pseudomonas syringae* pv. *syringae* on pear under controlled environment conditions. European Journal of Plant Pathology 104, 171–180.

Muthukumar, A., Thangavel, S., Ramasamy, N., Kaliyaperumal, S., Udhayakumar, R., 2019. Bio efficacy and phytotoxicity evaluation of TWG1 70WG (Thiophanate methyl) against Anthracnose disease in grapes. Journal of Emerging Technologies and Innovative Research, 516–524.

Nene, Y.L., Thapliyal, P.N., 1979. Fungicides in plant disease control. 2nd ed. Oxford and IBH pub. Co., New Delhi.

Ntasiou, P., Kerou, A.K., Karamanidou, T., Vlachou, A., Tziros, G., Tsouknidas, A., Karaoglanidis, G., 2021. Synthesis and characterization of novel copper nanoparticles for the control of leaf spot and anthracnose diseases of olive. Nanomaterials 11, 7.

Panek, M., Reinprecht, L., Hulla, M., 2014. Ten essential oils for beech wood protection - efficacy against wood-destroying fungi and moulds, and effect on wood discoloration. Bioresources 9, 5588–5603.

Rajendraprasad, M., Vidyasagar, B., Devi, G., Rao, S., 2017. *In vitro* evaluation of fungicides and biocontrol agents against *Rhizoctonia solani* in tomato. International Journal of Plant and Soil Science 17, 1–9.

Rama, J., Mallo, N., Biddau, M., Fernandes, F., Miguel, T.D., Sheiner, L., Choupina, A., Lores, M., 2020. Exploring the powerful phytoarsenal of white grape marc against bacteria and parasites causing significant diseases. Environmental Science and Pollution Research, 1–9.

Rampersad, S., Teelucksingh, L.D., 2012. Differential responses of *Colletotrichum gloeosporioides* and *C. truncatum* Isolates from different hosts to multiple fungicides based on two assays. Plant disease 96(10), 1526–1536.

Roberto, S.R., Youssef, K., Hashim, A., Ippolito, A., 2019. Nanomaterials as alternative control means against postharvest diseases in fruit crops. Nanomaterials, 9.

Shirsole, S., Khare, N., Lakpale, N., Kotasthane, A., 2019. Evaluation of fungicides against *Sclerotium rolfsii Sacc*. Incitant of collar rot of chickpea. The Pharma Innovation Journal 8, 310–316.

Teaca, C., Rosu, D., Mustața, F., Rusu, T., Rosu, L., Rosca, I., Varganici, C., 2019. Natural bio-based products for wood coating and protection against degradation: A review. Bioresources 14, 4873–4901.

Thiery, D., Louapre, P., Muneret, L., Rusch, A., Sentenac, G., Vogelweith, F., Iltis, C., Moreau, J., 2018. Biological protection against grape berry moths. A review. Agronomy for Sustainable Development 38, 1–18.

Vielba-Fernandez, A., Polonio, A., Ruiz-Jimenez, L., de Vicente, A., Perez-Garcia, A., Fernandez-Ortuno, D., 2020. Fungicide resistance in powdery mildew fungi. Microorganisms 8(9), 1431.

Vincent, J.M., 1947. Distortion of fungi hyphae in the presence of certain inhibitors. Nature 159, 850.

Vinothini, K., Devi, P., Prakasam, V., 2014. Evaluation of bio-efficacy of new fungicide molecule - UPF 509 (Azoxystrobin 8.3 w/w +Mancozeb 64.75 w/w) and biocontrol agents against powdery mildew of grapes. Pest Management in Horticultural Ecosystems 20, 75–79.

Yogev, E., Sadowsky, A., Solel, Z., Oren, Y., Orbach, Y., 2006. The performance of potassium phosphite for controlling *Alternaria* brown spot of citrus fruit. Journal of Plant Diseases and Protection 113, 207–213.