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An experiment was conducted with the objective to find out the compatibility of novel copper (NC101 and NCP102) and phosphonate (PN103 and PMN104) based fungicides with different antagonists used as biocontrol agents in grapes. Fungicides at different concentrations were screened against thirteen biocontrol agents which included seven isolates of *Trichoderma* sp., *Bacillus subtilis*, *Bacillus licheniformis, Pseudomonas fluroscens* and 3 bioinsecticides viz. *Beauveria bassiana,Verticilium* sp. and *Paecilomyces* sp. Experiment was carried out at ICAR-National Research Centre for Grapes, Pune. All the *Trichoderma* species were nearly compatible to both the copper and phosphonate based fungicide. Bacterial biocontrol agents namely *Bacillus subtilis, Bacillus licheniformis* and *Pseudomonas fluroscens* were found to be totally non compatible to all the novel fungicides. However entomopathogenic fungi *Beauveria bassiana, Verticillium lecanii* and *Paecilomyces lilacinus* showed mixed responses for both the formulations. *Beauveria bassiana* was completely inhibited by fungicide PN103 and PMN104 whereas inhibition of *Verticillium lecanii* was more than 60% at higher concentrations in phosphonates, *Paecilomyces lilacinus* was completely inhibited by fungicide PMN104 at 3000 and 4000 ppm. All the chemicals exhibited decreased tendency of compatibility with increase in concentration. The phytotoxicity effects of the novel fungicides on different varieties of grapes were found to be negative and, therefore the novel fungicides can be considered safe for use.

Key words: Biocontrol agents, compatibility, copper, grapes, phosphonates, phytotoxicity

INTRODUCTION

Grape is cultivated as an important commercial fruit crop in India. It covers an area of 123 thousand hectares occupying 2.01 % of the total area. India is also a major exporter of fresh grapes to the world. It has exported 193,690.55 MT of grapes worth of Rs.2,176.88 crores/ 298.05 USD Millions during the year 2019-20 (Kanitkar *et al*., 2020).

Production of grapevines is threatened by biotic (viruses, bacteria, fungi and insects) and abiotic stresses (i.e. drought, winter cold). Biotic stresses such as fungal infections reduce the yield and damage fruit and wine quality. Fungal diseases viz., downy mildew [*Plasmopara viticola* (Berk and Curtis) Berlese and De toni], powdery mildew (*Uncinula necator* (Schw.) Burn] and anthracnose [*Gloeosporium ampelophagum* (Pass) Sacc. (Perfect stage: *Elsinoe ampelina* (DeB) Shear)] are found to be the major constraints in grapevine cultivation (Muthukumar *et al.* 2019).

Application of fungicides is the most effective mode of disease control, but voluminous application of fungicides results in environmental problems as well as detection of pesticide residues. Integrated pest management strategy (IPM) is an important approach for control of diseases and pest in which use of pesticide is reduced by employing more biological control agents and maintaining good agricultural practices. This approach can be successful only if there is compatibility between the biological and non-biological components of the IPM (Saha *et al.* 2020). In viticulture, the biocontrol agents used largely includes *Trichoderma sp.,*

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Bacillus subtilis, Bacillus licheniformis, Pseudomonas fluorescens and entomopathogens such as Verticillium *lecanii, Beauveria bassiana* and *Paecilomyces lilacinus*. They inhibit the pests through the process of antagonism, competition and parasitism.

Copper as fungicide acts as a broad-spectrum 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. However phosphonate fungicides are unique in their ability to reduce some diseases 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 are effective at times on Pythium root rot and powdery mildew as well as some bacterial leaf spots. Phytotoxicity is the ability of pesticides to cause temporary or permanent damage to vegetative or generative organs, to reduce or totally inhibit germination, and to cause other physiological and morphological changes in sensitive plant species. It also depends on its variety or growth stage, or the compatibility of components in pesticide mixtures. Damage occurs in various ways, mostly as a chlorosis or necrosis (burn). (Vukoviæ, 2011).

Therefore, the present study was conducted to assess the compatibility of four novel fungicides namely NC101, NCP102, PN103 and PMN104 with different biological control agents (BCA) used in grape farming and alsoto check its phytotoxicity effects on different grape varieties.

MATERIAL AND METHODS

Fungicides

Four novel fungicides considered for the study were; NC101 (Contains modified copper particles), NCP102 (NC101 impregnated with phyto-hormones), PN103 (Phosphonate with mixture of macro and micro-nutrients), PMN104 (PN103 with additional micro nutrients). For evaluation of compatibility of novel fungicides at *in vitro* condition, NC101 (2000, 3000 and 4000 ppm), NCP102 (1000, 2000 and 3000 ppm), PN103 (2000, 3000 and 4000 ppm) and PMN104 (2000, 3000 and 4000 ppm) at three different concentrations were evaluated.Following concentrations were taken that is NC101 @ 8000 ppm, NCP102 @ 6000 ppm, PN103 @ 8000 ppm and PMN104 @ 8000 ppm were studied for phytotoxicity on different varieties of grapes.

Biocontrol agents

Ten antifungal agents;*Trichoderma asperelloides, Trichoderma afroharzianum, Bacillus subtilis DR39, Bacillus licheniformis TL 171, Pseudomonas fluorescens* and five native*Tricoderma* sp. isolated from different locations of Maharashtra and Tamil Nadu along with three entomopathogens namely *Verticillium lecanii*, *Beauveria bassiana* and *Paecilomyces lilacinus* were used for the study. All the BCAs were procured from culture collection of ICAR- National Research Centre for Grapes, Pune, India. Fungal strains were cultured and preserved on Potato Dextrose Agar (Hi Media MH096) and bacterial cultures were maintained on Nutrient Agar (Hi Media MM012).

Grape varieties used for phytotoxicity study

Total 13 varieties of grapes including both white (Maruti seedless, 2A- Clone,Thompson Seedless, Tas-A-Ganesh, Sonaka original, Manik Chaman, Manjari Naveen) and coloured (Fantasy Seedless, Manjari Shyama, Manjari Medika, Sarita Seedless, Red Globe, Crimson Red) varieties were tested and observed for the phytotoxic effect of the fungicides.

In vitro compatibility of novel fungicides with fungal biocontrol agents

Fungal biocontrol agents were tested for compatibility by following Poison food technique (Dhingra and Sinclair 1995).The quantified fungicide was mixed with the molten potato dextrose agar (PDA). Approximately 20 ml of poisoned medium was poured into sterilized Petri plates (90 mm) and after solidification they were seededcentrally with a fungal disc of 5 mm diameter cutfrom actively growing plate aseptically with the help of a sterilized corkborer. Unamended media served as control. Theplates were incubated at 28 ±1°C for5 days. The radial growth was measured after the maximum growth attainment was observed in control. Percent growth inhibitionwas calculated using the Vincent (1947) formula:

Percent growth inhibition (I)= C-T/C×100 where, I = Percent growth inhibition,T= Fungal colony diameter with treatment, C= Fungal colony diameter in untreated control.

In vitro compatibility of novel fungicides against bacterial biocontrol agents

The bacterial bio control agents were tested for its compatible nature with novel fungicides by using the modified technique of bacterial spot inoculation.

Desired concentrations of the fungicides were added to 200 ml sterilized, molten Nutrient Agar (NA) medium in 250 ml conical flask, mixed well and poured in sterilized 90 mm Petri dishes at the rate of 15-20 ml per plate. Five replicates for each fungicide were taken along with untreated control. A sterilized loopful of bacterial culture was taken aseptically from five days old culture and inoculated by making a circular spot of diameter 3mm at the centre of the plate. The inoculated plates were kept at 29 ± 1°C for 5 days. The observations on growth of bacterial species on media containing concentrations of test chemicals were recorded and percentage growth inhibition was calculated using the following formula given by Vincent (1947):-

$$L = C-T/C \times 100$$

where L is percentage of inhibition; C is radial growth of the bacterial species in control; T is radial growth of the bacteria in the presence of the fungicides.

Table 1. Phytotoxicity Rating Scale (PRS) **(**ppqs.gov.in/divisions/cib-rc/guidelines**)**

Crop response/ Crop injury	Rating
0-00%	0
1-10%	1
11-20%	2
21-30%	3
31-40%	4
41-50%	5
51-60%	6
61-70%	7
71-80%	8
81-90%	9
91-100%	10

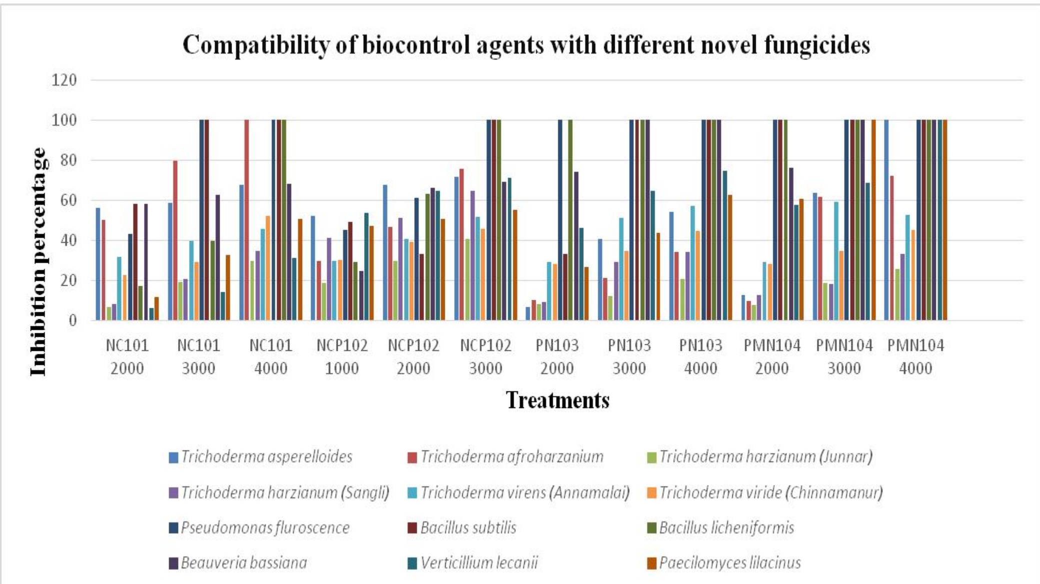

Fig.1: Compatibility of biocontrol agents with different novel fungicide

Phytotoxicity of novel fungicides in grape varieties

Phytotoxicity field trial was conducted, at ICAR-NRCG Pune. The experimental field consisted of grape vines in which two rows of each variety were tested for the phytotoxicity effect of the novel fungicides taken into account with different concentrations. Five replications for each treatment and its given concentrations were

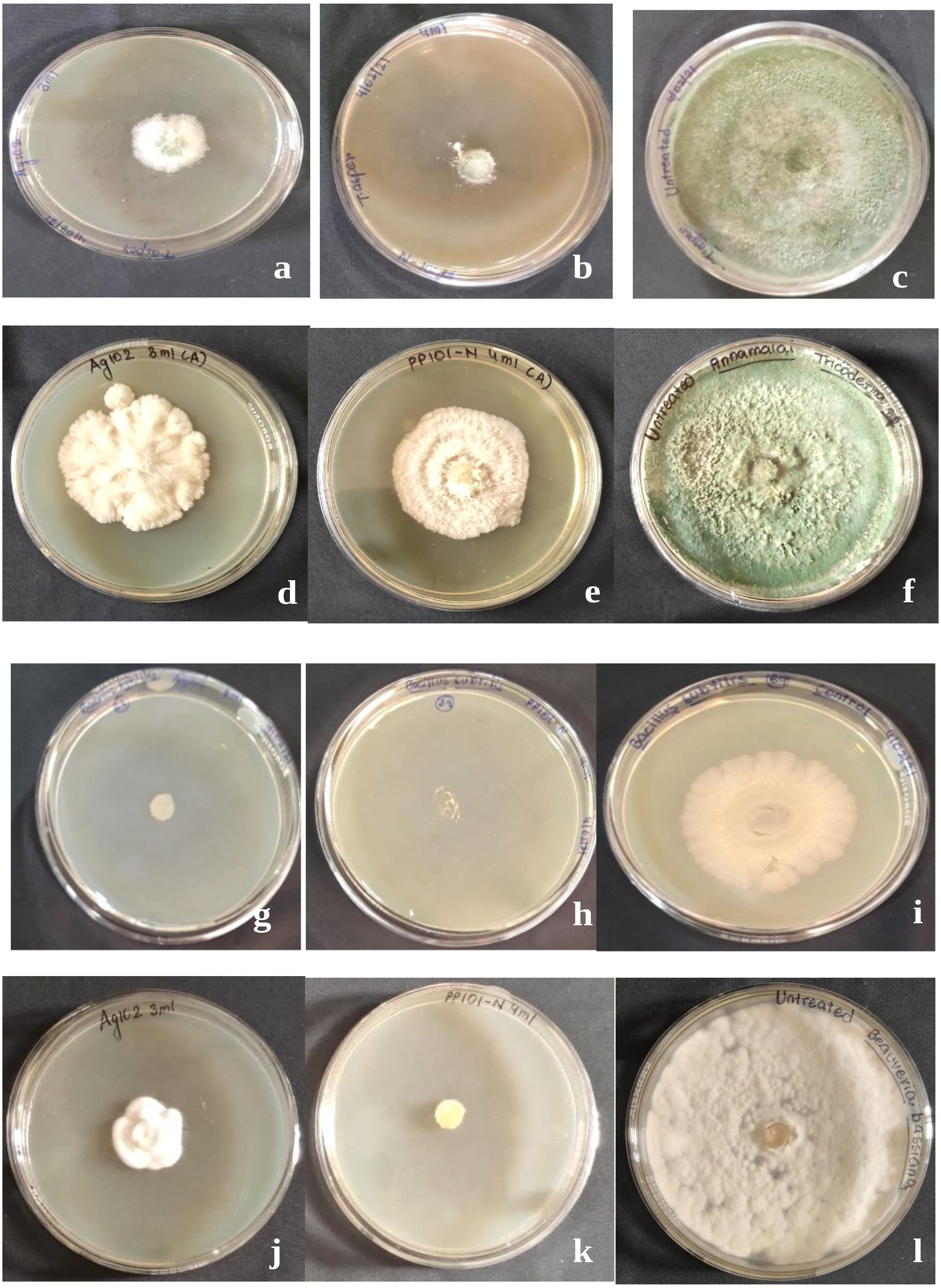

Fig. 1; Compatibility of *Trichoderma asperelloides*with (a) NCP102 3000 ppm (b) PMN104 4000 ppm (c) Untreated control. Compatibility of *Trichoderma virens*with (d) NCP102 3000 ppm (e) PMN104 4000 ppm (f) Untreated control. Compatibility of*Bacillus subtilis* (g) NCP102 3000 (h) PMN104 4000 ppm (i) Untreated control. Compatibility of*Beauveria bassiana* (j) PN103 1000 ppm (k) PMN104 4000 ppm (l) Untreated control sprayed with the help of handheld sprayer. The sprayed leaves of the given varieties were observed at 0, 1,3,5,7 and 10 days after the spray. The leaves were monitored for the presence of phytotoxic effect such as chlorosis, tip burning, necrosis, epinasty, hyponasty, vein clearing, curling, crinkling or cupping of the leave and the visual ratings were given in 0-10 scale as detailed in Table 1.

Statistical Analysis

The data were analysed in Completely Randomised Design (CRD) with Analysis of Variance (ANOVA) using SAS (ver. 9.3; SASInstitute Inc., Cary, North Carolina, USA) for compatibility study. Meanswere compared by the Tukey's test ($P < 0.05$).

RESULTS AND DISCUSSION

In the current study, various isolates of *Trichoderma* were examined for their compatibility with the novel fungicides taken into account. *Trichoderma* spp. are the most effective biocontrol agents against several diseases of grapes and are reported to show a higher tolerance to fungicides. The results manifested that *Trichoderma asperelloides* (Fig 1) and *Trichoderma afroharzanium* both were found to be most compatible with PN103 @ 2000, 3000 and 4000 ppm followed by PMN104 which showed highest compatibility at 2000 ppm, slightly compatible at 3000 ppm and non-compatible at 4000 ppm. For copper based fungicides NC101 and NCP102 it was a clear testimony that the both the chemicals were compatible at least concentration i.e. @ 1000 and 2000 ppm. However fungicide NCP102 was observed to be slightly compatible at 2000 and 3000 ppm. Similarly Dhanya *et al.* (2016) and Shahida *et al.*(2010) reported that *T. harzianum* and *T. viride* exhibited more than 60% and 100% inhibition by copper oxychloride and Bordeaux mixture respectively. Veena and Anandraj (2006) reported that Potassium phosphonate was not inhibitory and highly compatible to *T. harzianum* which was in parallel to the present findings.In the observations recorded in case of *Trichoderma* sp. of different locations in Maharashatra and Tamil Nadu both the Phosphonates PN103 and PMN104 were noted to be compatible at all concentrations (2000, 3000 and 4000 ppm) with less than 50% inhibition. Similarly the copper based fungicide NC101 at all concentrations (2000, 3000 and 4000 ppm) was also found to be compatible with the native isolates from different locations of Maharashtra and Tamil Nadu. NCP102 detected more than 60% inhibition at higher concentrations for *Trichoderma viride* (2000 and 3000 ppm) and *T. harzianum* @ 3000 ppm (Fig.1). The results were in agreement with the recent findings of Maheshwary *et al.* (2020) and Nandini *et al.* (2018) who reported that *Trichoderma* sp.was compatible with copper oxychloride and copper hydroxide.

Louis *et al.* (2016) and Nandini *et al.* (2018) reported copper oxy chloride and Bordeaux mixture were incompatible with bacterial bioagent *Pseudomonas fluorescens*. The present study was found in accordance with the above results as *Pseudomonas fluorescens* was found to be incompatible with the copper based fungicide NC101 at 3000 and 4000 ppm and compatible at 2000 ppm. Similar was the case with chemical NCP102 which was also found to be non-compatible with bacterial bio control agent at 2000 and 3000 ppm and compatible at 1000 ppm. The present study also revealed that both *Bacillus subtilis* (Fig 1) and *Bacillus licheniformis* were detected to be compatible at minimum concentration with NC101 (2000 ppm) and NCP102 (1000 and 2000 ppm) and non-compatible at higher concentrations. The results of present investigation were in conformity with the compatibility study of Manva *et al.* (2019) where the growth of *Bacillus* sp. was inhibited with double dosage of the chemical copper oxychloride. However both the phosphonates PN103 and PMN104 were found to be incompatible with *P. fluorescens*, *Bacillus subtilis* and *Bacillus licheniformis* at all concentrations (2000, 3000 and 4000 ppm) (Fig.1). Present findings were in contradiction with the earlier findings of Dhanya *et al.* (2016) where *Pseudomonas fluorescens* was observed to be compatible with potassium phosphonate.

In case of *Beauveria bassiana* (Fig 2) growth inhibition by NC101 was around 58.06 % at 2000 ppm which increased to 68.06% at 4000 ppm. NCP102 exhibited 25% inhibition at 1000 ppm and 69% inhibition at 3000 ppm. In case of phosphonates PN103 and PMN104 inhibition percentage was noted to be more than 70% at 2000 ppm and complete inhibition at 3000 and 4000 ppm. The present study was partly in contradiction with earlier studies of Kouassi *et al.* (2003) who reported incompatibility of *B. bassiana* with copper oxide. González *et al.* (2012) reported the effect of six fungicides on the entomopathogenic fungus *Lecanicillium (Verticillium) lecanii* (Zimm) where cuprous oxide at 10, 500, 1000 and 2000 mg kg^{-1} caused higher inhibition of the growth than metalaxyl, zineb and mancozeb at similar concentration which is partly in contradiction to the present findings. Fungicide NC101 was found to be compatible at all concentrations but the other copper based fungicide NCP102 was found to be slightly compatible at 2000 and 3000 ppm (Graph 1). On the other hand PN103 and PMN103 showed less than 60% inhibition at 2000 ppm but at 3000 and 4000 ppm inhibition percentage was more than 60%.In the present study, results of the nature of compatibility of the fungus *Paecilomyce* ssp. with different novel fungicide revealed that both the copper based fungicide NC101 and NCP102 were compatible with the entomopathogenic fungi at all concentrations. Fungicide PN103 was observed to be compatible at 2000 and 3000 ppm but PMN104 was found slightly compatible to incompatible at 2000, 3000 and 4000 ppm. The results were in accordance with the findings of Avery *et al.* (2013) which reported the compatibility of *Paecilomyce* sp. with various agrochemicals including fungicide based on borax and copper.

In the present study no phytotoxity symptoms like chlorosis, necrosis, scorching, epinasty, hyponasty and stunting or wilting was observed in the experiment under any treatment on different grape varieties. Hence the novel fungicides taken into account can be considered safe for use in grape vines. The results are in consistent with the findings of Provenzano *et al.* (2010) which reported no phytotoxicity in grape leaves with application of copper. However it is in contradiction with the earlier study conducted where the leaves showed visible signs of chlorosis due to phytotoxicity on copper application (Pessanha *et al.,* 2010).

CONCLUSION

The findings clearly highlighted the variable response in compatibility by the biocontrol agents to the novel fungicides NC101, NCP102, PN103 and PMN104. The bacterial biocontrol agents were not at all compatible to both the copper and phosphonate formulations. While *Trichoderma* sp. were relatively compatible to both. In case of entomopathogenic fungi, phosphonates inhibited *Beauveria bassiana* while the copper formulations had a mixed response. Similar was the case with *Lecanicillium (Verticillium) lecanii* (Zimm) where copper formulations were drastically opposite in their responses. The phosphonates however were incompatible to *Lecanicillium.* The copper formulations were compatible to *Paecilomyce s*sp., but the phosphonates showed mixed responses. The phytotoxicity effect was negative on different grape varieties. Field studies will be conclusive regarding the compatibility of bio control agents with the novel chemicals.

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