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Evaluation of compatibility of various chemical fungicides with *Cordyceps militaris* (L.) Link

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ABSTRACT

The increased use of chemical fungicides to combat plant pathogens is creating a negative impact on the environment. To overcome this concern, Cordyceps militaris a well-known entomopathogen was explored for its mycoparasitic ability against plant pathogens. In this regard, the compatibility of C. militaris was tested against various group of fungicides at systemic (0.075%, 0.050%, 0.100% and 0.150%) and non-systemic doses (0.25%, 0.50%, 0.75% and 1.00%) to assess whether they can effectively coexist or be used in combination with fungicide treatments in integrated disease management scheme. It was observed that C. militaris showed the highest compatibility with copper oxychloride with the least mycelial growth inhibition (15.34±0.53%) even at the highest concentration (1.00%). Among systemic fungicides, azoxystrobin and metalaxyl were found to be compatible with C. miltaris only at lower concentrations upto 0.25 percent and showed a 100 percent inhibition of mycelial growth at a concentration more than 0.25 percent whereas carbendazim was incompatible at all the tested concentrations.

Keywords: *Cordyceps militaris, compatibility, systemic fungicides, non-systemic fungicides*

Introduction

In modern agriculture, the use of chemical fungicides remains a common practice to combat fungal pathogens that pose threats to crop yields. However, the indiscriminate use of chemical fungicides has raised concerns about their negative impacts on the environment, non-target organisms, and the development of resistance in fungal populations. Approximately 110 novel fungicides have been produced over the past century enabling a rise in food production worth USD 12.8 billion annually in the US [1]. But nowadays concerns are being raised about the residual effect of these chemicals over the environment and living beings [2]. As a result, there is a growing emphasis on integrating sustainable and environment friendly pest management strategies such as the utilization of biological control agents.

Cordyceps militaris is a well-known entomopathogenic fungus that garnered significant attention for its medicinal and economic value because of the presence of pharmacologically active compounds and potential applications in various industries. This fungus parasitizes insect hosts and subsequently forms a fruiting body, known as the "caterpillar fungus," which has been widely used in traditional Chinese medicine for its purported health benefits [3]. In addition to its medicinal uses, *C. militaris* is increasingly being explored for its potential in biological control as an alternative to chemical pesticides, owing to its ability to regulate pest populations through natural mechanisms. Few of them are studied against plant pathogens. Among them, *O. sobolifera* was found to inhibit *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *C. miyabeanus* [4] and also *C. capsici* and *C. gloeosporioides* [5] while *Cordyceps dipterigena* showed antifungal activity against *Gibberella fujikuroi* [6] and *O. sinensis* was used against *Fusarium* spp. [7] and *Colletotrichum musae* [8]. Jaihan *et al.* (2016) [9] reported the antifungal activity of *Cordyceps* sp. against *Colletotrichum* sp. These studies have revealed promising results, demonstrating the ability of mushrooms to suppress the growth of pathogenic microorganisms and mitigate plant diseases in controlled laboratory conditions and field trials.

An unpublished study conducted by us regarding the *in vitro* mycoparasitic ability of *C. militaris* against *Colletotrichum capsici* and *Alternaria solani* showed that *C. militaris* as a potential biocontrol agent against these pathogens. Despite the promising potential of *C. militaris* as a biocontrol agent, there exists a significant research gap concerning its compatibility with commonly used fungicides. The interaction between *C. militaris* and fungicides, particularly those employed for disease management in agricultural settings remains inadequately understood. Addressing this research gap is crucial to ensure the effective integration of *C. militaris* into integrated disease management (IDM) programs without compromising its biocontrol efficacy or unintentionally causing harm to non-target organisms. So, a study aimed to bridge the knowledge gap by evaluating the compatibility of various fungicides with *C. militaris*. Through a series of laboratory experiments, we seek to assess the impact of fungicides on the growth and development of *C. militaris*. Ultimately, this investigation contributes to the development of environmentally sound agricultural practices that promote both pest control and ecological balance.

Material and Methods

Collection and maintenance of fungal culture

Cordyceps militaris fungal culture was purchased from the Directorate of Mushroom Research (DMR), Solan, Himachal Pradesh and subcultured in potato dextrose agar or Potato Dextrose Broth (PDB) and incubated at 25 °C.

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Evaluation of compatibility with different fungicide groups

The compatibility of *C. militaris* with commonly used fungicides for plant disease management was assessed in the Department of Plant Pathology, N. M. C. A., Navsari Agricultural University, Navsari by testing the tolerance of *C. militaris* to the fungicides used.

Fungicides belonging to different groups (Table 1) including contact and systemic fungicides were selected and kept in UV light for 30 minutes for surface sterilization. Molten potato dextrose agar was prepared and these fungicides were added in various concentrations of systemic fungicides *viz.,* 0.075 percent, 0.050 percent, 0.100 percent and 0.150 percent and various concentrations of non-systemic fungicides *viz.,* 0.25 percent, 0.50 percent, 0.75 percent and 1.00 percent. Then, this mixture was poured into Petri plates and allowed for solidification. After solidification, 9 mm mycelial discs of *C. militaris* taken from the periphery of fully grown culture was inoculated in these plates and incubated at 25 °C. The PDA plate without any fungicide served as a control.

The growth of the mycelia was observed and compared with the control and Percent Growth inhibition was calculated using the formula given by Vincent (1947) [10].

$$PGI = \frac{\text{Growth of Control-Growth of Treatment}}{\text{Growth of Control}} x100$$

By using this PGI, the tolerance of *C. militaris* to each group of fungicide at various concentrations was observed.

Table 1: List of fungicides used in the study.

Sr. No.	Name of the fungicide
1	Wettable sulfur 80% WP
2	Carbendazim 50% WP
3	Copper oxychloride 50% WP
4	Chlorothalonil 75% WP
5	Azoxystrobin 23.3% SC
6	Metalaxyl 35% WP

Statistical Analysis

The experiment was carried out in a Completely Randomized design (CRD) with three repetitions and the Least Significant Difference (LSD) was used for testing the treatment significance using statistical package for Social Sciences Version 22.0 (SPSS 22.0) program. Data were compared with LSD at $p \leq 0.05$.

Results

The compatibility of *C. militaris* was tested with six different fungicides including both systemic (carbendazim 50% WP, azoxystrobin 23.3% SC and metalaxyl 35% WP) and non-systemic (wettable sulfur 80% WP, chlorothalonil 75% WP and copper oxychloride 50% WP) groups with two different set of concentrations *viz.,* recommended doses of systemic fungicide (0.075%, 0.050%, 0.100% and 0.150%) and recommended doses of non-systemic fungicides (0.25%, 0.5%, 0.75% and 1.00%). The data are presented in Tables 2, 3 and Fig. 1, 2.

Compatibility of *C. militaris* with Different Fungicides at the Recommended Doses of Systemic Fungicides

The data pertaining to the compatibility of *C. militaris* with various groups of fungicides are depicted in Table 2 and Fig. 1. *C. militaris* was found to be compatible with all the fungicides at all concentrations except carbendazim 50% WP where the fungi showed highest level of susceptibility at all the tested concentrations. It is also noticed that the contact fungicides are much safer than the systemic fungicides. The highest mycelial growth (63.00±0.00 mm) was observed in the control. The growth inhibition of mycelia of *C. militaris* ranged from 4.76±0.92 percent in copper oxychloride 50% WP up to 100.00±0.00 percent in carbendazim 50% WP.

Among different treatments investigated, the highest level of tolerance with the least inhibition percentage (4.76±0.92%) was observed in copper oxychloride 50% WP at the concentration of 0.075 percent with a mycelial diameter of 60.00±0.58 mm. This was significantly the highest mycelial diameter observed as compared to other treatments.

The next best treatment in terms of good mycelial growth was copper oxychloride 50% WP at a concentration of 0.050 percent with a mycelial diameter of 57.00±0.58 mm. This was followed by copper oxychloride 50% WP at the concentration of 0.100 percent and wettable sulfur 80% WP at the concentration of 0.075 percent with a mycelial growth of 55.10±0.15 mm and 55.00±0.58mm, respectively.

Chlorothalonil 50% WP at all the tested concentrations showed a moderate level of compatibility with *C. militaris* with an inhibition of mycelial growth of 41.80±1.40 percent over control at the highest concentration of 0.150 percent whereas, wettable sulfur 80% WP showed a moderate level of compatibility with *C. militaris* with inhibition of mycelial growth of 31.22±0.53 percent and 34.39±0.53 percent over control at the highest concentrations (0.100% and 0.150%) tested.

Among the systemic fungicides, the highest compatibility (35.67±0.88 mm) was exhibited by metalaxyl 35% WP at the concentration of 0.075 percent with the percent growth inhibition of 43.39±1.40 percent. This was followed by the mycelial growth (33.33±0.88 mm) observed in the treatment with metalaxyl 35% WP at the concentration of 0.050 percent with a growth inhibition of 47.09±1.40 percent. The least compatibility with 100 percent growth inhibition over control was observed in carbendazim 50% WP in all the tested concentrations.

The Fig. 1 pronounced the percent compatibility of each fungicide with *C. militaris* irrespective of the concentrations at the recommended dose of systemic fungicides. It is clearly noticeable that the highest level of compatibility was exhibited by copper oxychloride 50% WP (28%) and the least compatibility (11%) and no compatibility by azoxystrobin 23.3% SC and carbendazim 50% WP, respectively showing the highest level of tolerance to copper oxychloride 50% WP and highest susceptibility to carbendazim 50% WP.

Compatibility of *C. militaris* with Different Fungicides at the Recommended Doses of Non-Systemic Fungicides

The data in Table 3 and Fig. 2 revealed the compatibility of various systemic and non-systemic fungicides at different recommended doses of non-systemic fungicides. The percent inhibition of mycelial growth of *C. militaris* over control was in the range of 15.34±0.53 percent in copper oxychloride 50% WP up to 100.00±0.00 percent in carbendazim 50% WP.

The significantly least mycelial growth inhibition (15.34±0.53%) with a significant maximum mycelial diameter (53.33±0.33 mm) was recorded in the treatment with copper oxychloride 50% WP at the concentration of 0.25 percent. This was followed by 29.63±0.53 percent and 32.28±0.53 percent inhibition observed in copper oxychloride 50% WP at the concentrations of 0.50 percent and 0.75 percent with the mycelial diameters of 44.33±0.33 mm and 42.67±0.33 mm. The next best treatment was wettable sulfur 80% WP with 33.86±1.06 percent growth inhibition and with a mycelial diameter (41.67±0.67mm) at the concentration of 0.25 percent. This was statistically at par with copper oxychloride 50% WP at the concentration of 1.00 percent with a percent inhibition and mycelial diameter of 34.39±0.53 percent and 41.33±0.33 mm, respectively. Wettable sulfur 80% WP and chlorothalonil 50% WP were moderately compatible with inhibition of 63.49±0.92 percent and 52.38±0.92 percent, respectively at the highest concentration (1.00%).

Among the systemic fungicides, metalaxyl 35% WP and azoxystrobin 23.3% SC showed compatibility only at their lowest concentration (0.25%) with a percent growth inhibition of 66.67±0.92 percent and 80.42±0.53 percent, respectively with mycelial diameters of 21.00±0.58 mm and 12.33±0.33 mm. These fungicides at concentrations more than 0.25 percent and carbendazim 50% WP at all the tested concentrations were found to be non-compatible with *C. militaris* with 100.00±0.00 percent growth inhibition.

The Fig. 2 revealed the percent compatibility of each fungicide with *C. militaris* irrespective of concentrations at the recommended dose of non-systemic fungicides. It is noticeable that the highest level of compatibility was exhibited by copper oxychloride 50% WP (38%) and the least compatibility (3%) and no compatibility by azoxystrobin 23.3% SC and carbendazim 50% WP, respectively showing the highest level of tolerance to copper oxychloride 50% WP and highest susceptibility to carbendazim 50% WP.

Discussion

This is the first ever study on evaluation of the compatibility of *C. militaris* with commonly used fungicides. In the present study, the compatibility of *C. militaris* was found to be concentration dependent exhibiting very good compatibility with non-systemic fungicides at all the tested concentrations. The systemic fungicides exhibited a good compatibility only at lower concentrations whereas at the higher concentration they retarded the growth of *C. militaris.* Carbendazim was found to be highly incompatible with C. *militaris* at all the tested concentrations.

Similar results were reported by [11], [12] and [13] who observed that *T. viride* was highly incompatible with carbendazim showing 100 percent inhibition of the mycelial growth even at recommended field concentration. Another study revealed that among eighteen fungicides studied, a 100 percent growth inhibition of *Trichoderma* spp. was observed with carbendazim, benomyl, carboxin, propiconazole, hexaconazole, tricyclozole, tridemorph, chlorothalonil whereas dinocap, copper oxychloride, fosetyl-Al, captan, thiram and metalaxyl showed a 70 percent inhibition of radial growth with least compatibility. While the highest compatibility was exhibited by wettable sulfur with the least inhibition of mycelial growth (2.2%) of *Trichoderma* spp [14]. While in another study, the compatibility of copper oxychloride with *T. viride* was reported [15]. Hence, the present study is more or less in tune with the previous studies.

Conclusion

The compatibility study on *C. militaris* ensured that the application of *C. militaris* along with a lower dose of systemic fungicides and recommended dose of non-systemic fungicide can be integrated to provide optimal disease management strategies in integrated disease management (IDM) scheme. As carbendazim was found to be incompatible, it is not recommended to apply with *C. militaris.*

Future scope of the study

As *C. militaris* is proved to be compatible with various group of fungicides, it can be used as a biocontrol agent against various plant pathogens and can be integrated with fungicides for precise management of plant diseases.

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Authors contribution: The study was carried out and the first draft of the manuscript was prepared by Ms. Saranya S under the technical guidance of Dr. Priya John. Both the authors did manuscript revision and prepared the final draft.

Conflict of interest: The authors declare that they have no conflict of interest. There are no human or animal participants involved in this research.

Table 2: Effect of different fungicides on the mycelial growth of C. militaris at the recommended doses of systemic fungicides

Sr. No.	Treatments	Concentration (%)	Mean diameter of mycelium (mm)	Percent inhibition of mycelial growth over control (%)
1	Wettable sulfur 80% WP	0.075	55.00±0.58[d]	12.70±0.92 (20.85±0.79)[n]
		0.050	50.67±0.88[e]	19.58±1.40 (26.23±1.01)[m]
		0.100	43.33±0.33[g]	31.22±0.53 (33.97±0.33)[jk]
		0.150	41.33±0.33[h]	34.39±0.53 (35.90±0.32)[ij]
2	Carbendazim 50% WP	0.075	0.00±0.00[p]	100.00±0.00 (90.00±0.00)[a]
		0.050	0.00±0.00[p]	100.00±0.00 (90.00±0.00)[a]
		0.100	0.00±0.00[p]	100.00±0.00 (90.00±0.00)[a]
		0.150	0.00±0.00[p]	100.00±0.00 (90.00±0.00)[a]
3	Copper oxychloride 50% WP	0.075	60.00±0.58[b]	4.76±0.92 (12.49±1.25)[q]
		0.050	57.00±0.58[c]	9.52±0.92 (17.94±0.90)[op]
		0.100	55.10±0.15[d]	12.70±1.83 (20.78±1.59)[n]
		0.150	52.33±0.88[e]	16.93±1.40 (24.26±1.08)[m]
4	Chlorothalonil 75% WP	0.075	47.67±0.88[f]	24.34±1.40 (29.54±0.93)[l]
		0.050	43.67±0.88[g]	30.69±1.40 (33.63±0.87)[k]
		0.100	40.00±0.58[h]	36.51±0.92 (37.17±0.55)[i]
		0.150	36.67±0.88[i]	41.80±1.40 (40.28±0.81)[h]
5	Azoxystrobin 23.3% SC	0.075	27.00±0.58[l]	57.14±0.92 (49.11±0.53)[ef]
		0.050	22.67±0.88[m]	64.02±1.40 (53.15±0.84)[d]
		0.100	20.00±0.58[n]	68.25±0.92 (55.71±0.56)[c]
		0.150	16.67±0.88[o]	73.54±1.40 (59.06±0.91)[b]

6	Metalaxyl 35% WP	0.075	35.67±0.88^{i}	43.39±1.40 (41.20±0.81)^{gh}
		0.050	33.33±0.88^{j}	47.09±1.40 (43.33±0.80)^{g}
		0.100	29.00±0.58^{k}	53.97±0.92 (47.28±0.53)^{f}
		0.150	25.33±0.88^{l}	59.79±1.40 (50.65±0.82)^{e}
7	Control	-	63.00±0.00^{a}	0.00±0.00 (0.00±0.00)^{r}
CV(%)			3.46	4.11

Data are means ± standard error of means in triplicates. Figures in parenthesis are angular transformed values.
Values in columns followed by the common letter(s) are not significantly different at 5% level of significance analysed using LSD method

Table 3: Effect of different fungicides on the mycelial growth of C. militaris at the recommended doses of non-systemic fungicides

Sr. No.	Treatments	Concentration (%)	Mean diameter of mycelium (mm)	Percent inhibition of mycelial growth over control (%)
1	Wettable sulfur 80% WP	0.25	41.67±0.67^{de}	33.86±1.06 (35.58±0.64)^{kl}
		0.50	40.00±0.58^{f}	36.51±0.92 (37.17±0.55)^{j}
		0.75	26.67±0.33^{i}	57.67±0.53 (49.41±0.31)^{e}
		1.00	23.00±0.58^{l}	63.49±0.92 (52.83±0.55)^{d}
2	Carbendazim 50% WP	0.25	0.00±0.00^{0}	100.00±0.00 (89.64±0.00)^{a}
		0.50	0.00±0.00^{o}	100.00±0.00 (89.64±0.00)^{a}
		0.75	0.00±0.00^{o}	100.00±0.00 (89.64±0.00)^{a}
		1.00	0.00±0.00^{o}	100.00±0.00 (89.64±0.00)^{a}
3	Copper oxychloride 50% WP	0.25	53.33±0.33^{b}	15.34±0.53 (23.06±0.42)^{n}
		0.50	44.33±0.33^{c}	29.63±0.53 (32.98±0.33)^{m}
		0.75	42.67±0.33^{d}	32.28±0.53 (34.62±0.32)^{l}
		1.00	41.33±0.33^{e}	34.39±0.53 (35.90±0.32)^{k}
4	Chlorothalonil 50% WP	0.25	35.67±0.33^{g}	43.39±0.53 (41.20±0.31)^{i}
		0.50	33.00±0.58^{h}	47.62±0.92 (43.64±0.53)^{h}
		0.75	31.33±0.88^{i}	50.27±1.40 (45.15±0.80)^{g}
		1.00	30.00±0.58^{j}	52.38±0.92 (46.37±0.53)^{f}
5	Azoxystrobin 23.3% SC	0.25	12.33±0.33^{n}	80.42±0.53 (63.74±0.38)^{b}
		0.50	0.00±0.00^{o}	100.00±0.00 (89.64±0.00)^{a}
		0.75	0.00±0.00^{o}	100.00±0.00 (89.64±0.00)^{a}
		1.00	0.00±0.00^{o}	100.00±0.00 (89.64±0.00)^{a}
6	Metalaxyl 35% WP	0.25	21.00±0.58^{m}	66.67±0.92 (54.74±0.56)^{c}
		0.50	0.00±0.00^{o}	100.00±0.00 (89.64±0.00)^{a}
		0.75	0.00±0.00^{o}	100.00±0.00 (89.64±0.00)^{a}
		1.00	0.00±0.00^{o}	100.00±0.00 (89.64±0.00)^{a}
7	Control	-	63.00±0.00^{a}	0.00±0.00(0.36±0.00)^{o}
CV(%)			3.08	1.60

Data are means ± standard error of means in triplicates. Figures in parenthesis are angular transformed values
Values in columns followed by the common letter(s) are not significantly different at 5% level of significance analysed using LSDPhoto 1: Compatibility of C. militaris with selected fungicides under in vitro method

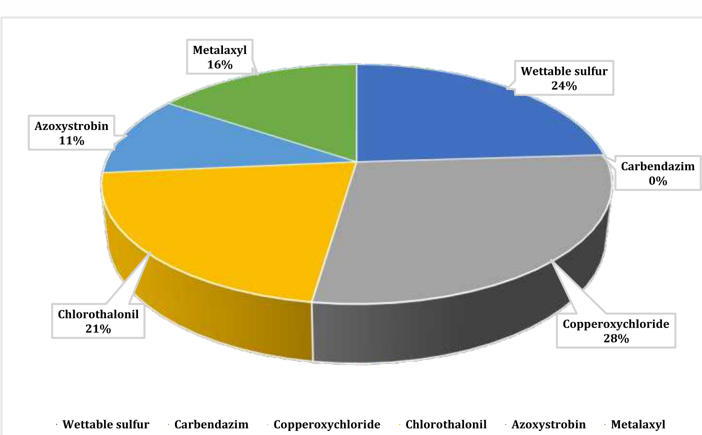

Fig. 1: Percent compatibility of various fungicides with C. militaris at recommended doses of systemic fungicides

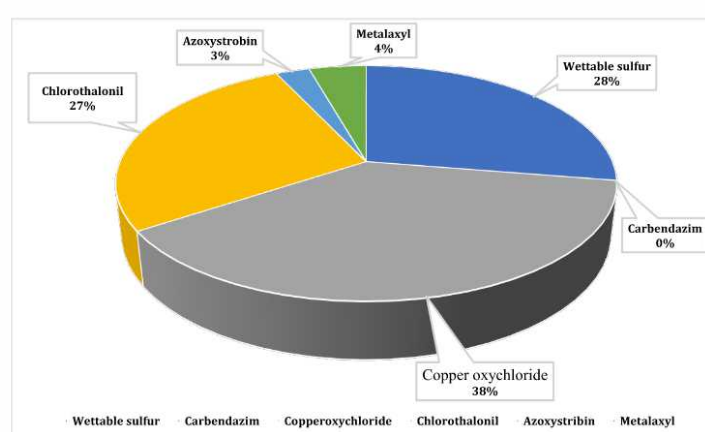

Fig. 2: Percent compatibility of various fungicides with C. militaris at recommended doses of non-systemic fungicides

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