DOI 10.12905/0380.sydowia77-2025-0069 Published online 5 August 2024

Applying *Cordyceps militaris* biopesticide to reduce *Brevicoryne brassicae* infestation of Brassica *oleracea* crops

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Bhatia I. & Oleksak M. (2024) Applying *Cordyceps militaris* Biopesticide to Reduce *Brevicoryne brassicae* Infestation of *Brassica oleracea* Crops. — Sydowia 77: 69-80.

Chemical ingredients in pesticides unintentionally cause 385 million annual cases of acute poisonings, resulting in 11,000 fatalities each year. An environmentally friendly alternative may exist in *Cordyceps militaris*, an entomopathogenic fungus traditionally used as a health supplement. This study was conducted to determine if a *C. militaris* spray would effectively control *Brevicoryne brassicae* (cabbage aphids), a prevalent agricultural pest within the *Aphididae* family. The experiment started with 3,064 aphids grown on 48 *Brassica oleracea* plants. An experimental group of 24 plants was sprayed with a solution of *C. militaris* and water while a control group of 24 plants was sprayed with water. *C. militaris* effectiveness was assessed by taking pictures of each plant on each day and counting the number of aphids on each plant to establish a daily count of aphid populations for both the control and experimental groups. Results showed that *C. militaris* exhibited a 91.93 % mortality rate on *B. brassicae* over 8 total days. This study suggests that an optimized biopesticide using *C. militaris* may effectively and safely prevent pest infestation.

Keywords: fungi, insecticide, mycopesticide, pesticide, pest control.

Commercial pesticides are integral to the agricultural industry due to their role in deterring pests that attack crops, thereby protecting the global food supply. With the global population expected to increase by an additional 1.8 billion by 2050, eliminating factors that compromise our existing food supply is essential to ensure human survival (United Nations 2019). Currently, these efforts are inadequate, as 40 % of annual crop production is lost to pests, resulting in $70 billion lost annually (Food and Agriculture Organization of the United Nations 2021). One especially prominent agricultural pest is the aphid, part of the *Aphididae* family. Aphids directly affect the health of agricultural crops by feeding on the phloem of their victim plants, thus stealing necessary nutrients for plant reproduction and growth. In addition to direct injuries, aphids inject phytotoxic saliva into victim plants and transmit 275 different viruses that severely compromise crop health to a greater degree than direct feeding on crops. As a result, damaged fruiting plants usually bear smaller fruit and other affected plants exhibit unobservable injuries such as decreased root proliferation and size. In 2010, aphids were responsible for annual losses of 700,000 tons of wheat, 850,000 tons of potatoes, and 2,000,000 tons of sugar beet. Additionally, 26 % of the 45 major insect pests that affect the 6 major global crops (maize, wheat, potatoes, sugar beet, barley, and tomatoes) belong to the *Aphididae* family. Because of this, they are considered to be of serious economic detriment, especially in temperate climates (Dedryver et al. 2010).

To combat this issue, pesticides are applied to deter and eliminate pests, with some being able to control between 90–100 % of an insect population (Sudo et al. 2019). However, problems arise when this level of toxicity begins to affect humans and the environment. Today's commercial pesticides pose a multitude of problems to health, such as deteriorating the structure of DNA in cells, which can ultimately lead to cancer (Reynolds et al. 2002, Hou et al. 2013), and causing developmental injury in embryos as a result of fewer blastocysts and increased rate of apoptosis (Greenlee et al. 2004). Moreover, pesticide drift can cause poisoning and illnesses in those who are in close proximity to farms (Roberts et al. 2003, Lee et al. 2011). Altogeth-

er, this toxicity is responsible for 11,000 fatalities among 385 million cases of unintentional, acute pesticide poisoning (Boedeker et al. 2020).

In recent years, attempts towards decreasing the harmful effects of pesticide usage involve: increasing the efficiency of pesticides, reducing the amount of pesticides necessary to kill a certain amount of pests, allowing for pest selectivity, making application safer for non-target organisms, higher biodegradability, reducing harm on the surrounding environment, controlled-release formulations, preventing unwanted release of harmful active substances, using derivatives of natural products, decreasing harm to the environment (Boh & Kornhauser 2003). Numerous additional studies have been published discussing the harmful effects of pesticides, indicating that these measures have not been developed or utilized enough to adequately mitigate the threats that pesticides pose to human health. New solutions are thus recommended to decrease public health risks caused by pesticides.

One of these new solutions exists in the field of entomopathogenic fungi, also referred to as mycopesticides, which serve to attack and eliminate insects for their own survival (Baja et al. 2020, Berestetskiy & Hu 2021, Han et al. 2014, Jordan et al. 2021, Kumari et al. 2022, Kryukov et al. 2020, Lee et al. 2018, Ou et al. 2019, Wu et al. 2021). Because these fungi specifically target insects, they can be used to deter pests in both agricultural and smaller-scale home gardening settings (Gul et al. 2014). Unlike commercial pesticides, some mycopesticides can be safely ingested by humans, proving that they are not hazardous to our health.

One such mycopesticide that can possibly be used to control pests is *Cordyceps militaris*. It is currently widely used as a health supplement and has been consumed for thousands of years for the health benefits it provides: inhibiting cell proliferation (preventing cancer), stimulating thrombolytic activity (decreasing risks presented by cardiovascular diseases), enhancing fertility, preventing melanogenesis, providing anti-oxidative, anti-inflammatory, anti-microbial, anti-cholesterol, and anti-diabetic properties (Mehra et al. 2017). Moreover, its ability to infect and control silkworm larvae has been extensively studied, and researchers have traced these entomopathogenic abilities to the chemical cordycepin (Kato et al. 2021) and the protein CMP (Bai et al. 2018). As displayed in Fig. 1, *C. militaris* additionally utilizes adhesion proteins that attach to insect cuticles (Wang et al. 2016). Using a series of physical and chemical processes, *C. militaris* creates an opening in the cuticle, allowing it to establish a pathway between the fungus and the insect. This allows the fungus to deactivate sen-

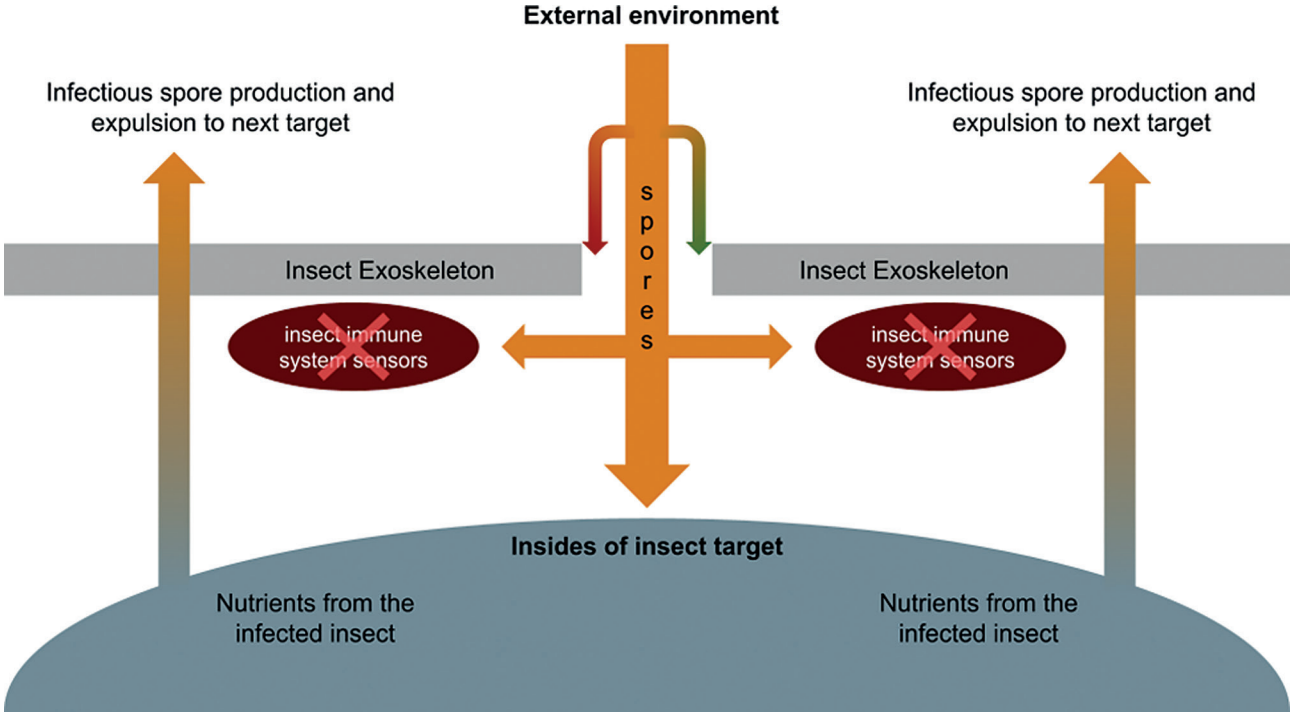

Fig. 1. Diagram of *C. militaris*' insecticidal mechanisms.

sors in the insect that would normally obstruct parasitic behavior and send spores to feed on nutrients inside the insect host. Once all the nutrients are depleted from the host, additional spores are colonized inside the host and later propelled into the surrounding environment (Gul et al. 2014).

Previous studies have been conducted demonstrating *C. militaris*' insecticidal properties, albeit limited to moths, butterflies, and ants (Jo et al. 2020, Ono et al. 2022, Prommaban et al. 2022, Woolley et al. 2020). Evaluating the potential application of *C. militaris* to the agricultural industry requires experimentation on, but not limited to, locusts, beetles, crickets, aphids, mosquitoes, and worms. This project aims to determine if the utilization of *C. militaris* as a spray solution for *Brevicoryne brassicae* infiltration of *Brassica oleracea* will verify its usage as an environmentally friendly and potentially cost-effective pesticide. It is predicted that a scheduled spray of *C. militaris* will be an effective solution to the problem of aphid insect infiltration of *B. oleracea* as a result of its physical and chemical means of eliminating insects.

Materials and methods

The key materials used in the experimentation are displayed in Tab. 1, including the purpose for why each material was required.

Procedure

The experiment involved a total of 48 red cabbage plants (cultivars of the *B. oleracea* species). These plants were placed into eight 1.5' by 1.5' by 1' bins with each bin housing 6 plants. 4 bins were assigned as the control group and the remaining 4 bins were assigned as the experimental group.

For experimental controls, all plants were watered daily and fertilized weekly during the growing process, and the placement of the bins ensured that each bin received equal amounts of sunlight throughout each day during the growing process. Due to rainy conditions, all 48 plants were transferred into a 12' by 8' by 7' greenhouse (Fig. 2).

Being the plants that aphids naturally grow on, the *B. oleracea* plants successfully attracted hundreds of aphids 4 weeks after they were grown. These aphids mainly fed on the apical buds and leaf undersides. 2 weeks later, the experiment was initiated. Throughout the experiment, the plants in the control group were sprayed with a water spray and the plants in the experimental group were sprayed with a *C. militaris* spray (for details, see Tab. 1).

The *C. militaris* spray was made using a 3:4 ratio of *C. militaris* fruiting bodies and tap water, giving the spray a concentration of 89.184 g/l of *C. militaris*. The *C. militaris* mushrooms were sourced from Far West Fungi. These ingredients were blended until the mixture was of a smooth consistency. The mixture was then filtered twice using a muslin cloth (one separate cloth for each filtration), and the liquid was distributed into multiple 12 oz spray bottles. The water spray was made by filling the same 12oz spray bottles with tap water acquired from the same faucet.

This experiment consisted of three spray periods within 8 days. During the spraying process, the bins were placed 2 feet apart from each other to avoid any contact or interference between the bins. Approximately 2.667 oz of solution, whether *C. militaris* and water or water only, was sprayed on each plant per spraying session, amounting to 384 oz total of solution used (192 oz *C. militaris* solution and 192 oz water solution). A chronological description of what occurred on each day is presented below:

Day 1: Introductory observation of control and experimental groups
Day 2: First spray and observation (water spray on control group, *C. militaris* + water spray on experimental group)
Day 3: Cooldown and observation - no spray period
Day 4: Second spray and observation (water spray on control group, *C. militaris* + water spray on experimental group)
Day 5: Cooldown and observation - no spray period
Day 6: Third spray and observation (water spray on control group, *C. militaris* + water spray on experimental group)
Day 7: Cooldown and observation – no spray period
Day 8: Conclusion of experiment

The experimental assessment involved three systems of visual documentation: baseline pictures and individual pictures. These pictures were taken on the Apple iPhone 13 Pro rear camera. The baseline pictures utilize Camera Frame 1, while the individual pictures utilize Camera Frame 2. Both the control group and experimental group were documented throughout the duration of the experiment. The baseline pictures were taken to showcase the overall health of the plants (i.e. if plant health declined or improved), whereas individual pictures were taken to establish a numerical documentation of the aphids.

1. Baseline pictures

a. Pictures of the top and 4 sides of each individual bin

Tab. 1. All materials used in the experiment.

Name of material used	Purpose
48 *B. oleracea* plants arranged in groups of 6, with each group placed in a bin, resulting in 8 total bins	The medium on which the aphids live and thus, on which the experiment will be conducted
Cabbage aphids (*B. brassicae*)	The insect used in the experiment that the *C. militaris* spray will be tested on
C. militaris spray	A spray consisting of a ratio of ¾ cup of *C. militaris* fruiting bodies blended with 1 cup of tap water; used on the aphids as a pesticide
Water spray	A spray consisting of tap water and compared against the *C. militaris* spray to determine the effectiveness of the *C. militaris* spray
Camera Frame 1	Used to take baseline pictures (refer to the definition of this term below) of the plants
Camera Frame 2	Used to take individual pictures (refer to the definition of these terms below) of the plants
iPhone 13 Pro	The physical camera used to take pictures on each day of the experiment

b. Pictures of the top and all 4 sides of the group of all bins (control and experimental)
c. The phone's position on Camera Frame 1 remained constant throughout the experiment for all bins.

2. Individual pictures

a. Pictures of individual plants in each bin.
b. The phone's position on Camera Frame 2 was established for each plant for the highest quality documentation possible, and this position remained constant throughout the experiment for all bins.

During the 8-day experiment, the plants were documented daily from 1:00 pm to 3:00 pm. The methods of documentation that were used on each day are listed below:

Day 1: Baseline only, Days 2, 3, 4,: Individual pictures, Day 5: Baseline, Individual pictures, Days 6, 7: Individual pictures, Day 8: Baseline only.

The individual pictures were used to create a visual representation of how the aphids were affected by the *C. militaris* and water sprays; the methods used to make these assessments are highlighted in Tab. 2. A folder system was created to organize these individual pictures by the day on which they were taken. Each picture corresponded to one plant, so by counting the number of live aphids in each picture, it was possible to determine the number of aphids present on each plant each day.

These counts were recorded on a spreadsheet organized chronologically, with each column corresponding to one day and each row corresponding to an individual plant. Days 1 and 8 were introductory and conclusion days that served to document the state of the plants and aphids rather than the number of aphids present on each group. As such, because the individual pictures were only documented on days 2 through 7, the recorded data represents a 6-day period within the experiment instead of the full 8-day experimental period.

Tab. 2. Aphid appearance and corresponding health.

Aphid appearance	Health status
Plump and grey	Live, healthy aphids before cordyceps spraying
Plump and light green	Live, healthy aphids before cordyceps spraying
Dark green	Dead or currently dying aphids after cordyceps spraying
Shriveled and grey	Dead aphids after cordyceps spraying

Results and discussion

Derived from the individual pictures, the aphid counts for each day of the experiment were transcribed onto Tab. 3, which shows the results of all 48 plants divided into 2 groups of 24 plants, where each group is divided into 4 groups of 6 plants to recreate the setup used in the actual experiment. Each plant is identified in the first column of the table, in which "ctr" represents the control group, "exp" represents the experimental group, the following number conveys the corresponding bin, and the last number conveys the corresponding plant within that specific bin. Each day, aphid counts

Fig. 2. *Brassica oleracea* growing in greenhouse.

were conducted before any sprays occurred. The survival rates for each group are also shown, established by dividing the total number of live aphids present on all plants within a certain group on one specific day by the total number of live aphids present on all plants within that same group at the beginning of the experiment. The mean aphid count for each group was utilized for statistical analysis.

The survival rates for the control group on each day exhibited an overall small decrease over the 6-day period from 100 % to 82.71 %. This behavior was not consistent throughout each day. From day 3 to day 4, there was an increase in survival rate from 91.03 % to 93.25 %, and from day 6 to day 7, there was an increase in survival rate from 81.99 % to 82.71 %. These increases can also be observed by taking the derivative of the survival rate (survival rate of the current day divided by the survival rate of the previous day), thus demonstrating the rate of change in survival rate. Any values above 100 % indicate an increase in aphid population, and any values below 100 % indicate a decrease in aphid population.

The survival rates for the experimental group exhibited a much stronger and more consistent decline over the 6-day period when compared to the control group, from 100 % to 8.07 %. Most of the decrease in survival rate was observed from day 2 to day 3, and the survival rate moderately but consistently declined afterwards. No increases in aphid survival were observed throughout the 6-day period, confirmed by all survival rate derivatives calculated below 100 %.

A comparison of the two groups was visually documented in Fig. 3. This demonstrated the much greater initial decline (30.84 % vs. 91.03 %) and subsequent declines in aphid population in the experimental group compared to the control group. It also showed a consistently downward trend in terms of aphid survival in the experimental group and

Tab. 3. Aphid counts and totals.

Plant #	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
ctr1 1	17	15	14	13	16	11
ctr1 2	24	32	27	23	26	24
ctr1 3	52	41	55	53	46	43
ctr1 4	85	83	64	80	61	58
ctr1 5	44	43	40	41	37	31
ctr1 6	3	3	2	1	2	4
ctr2 1	12	12	12	12	10	8
ctr2 2	161	146	148	151	153	146
ctr2 3	38	38	49	39	39	41
ctr2 4	222	217	194	185	184	181
ctr2 5	290	254	281	279	258	263
ctr2 6	13	15	6	28	13	36
ctr3 1	46	30	54	34	37	35
ctr3 2	49	38	44	39	42	41
ctr3 3	71	59	70	62	61	58
ctr3 4	104	89	98	70	75	76
ctr3 5	52	32	35	29	21	20
ctr3 6	50	48	43	44	44	43
ctr4 1	12	14	10	11	8	9
ctr4 2	33	32	25	17	10	11
ctr4 3	22	27	14	18	12	25
ctr4 4	13	12	18	10	19	19
ctr4 5	86	84	90	69	53	55
ctr4 6	28	26	31	38	25	25
Ctr. group survival rate	100.00 %	91.03 %	93.25 %	88.15 %	81.99 %	82.71 %
Survival rate derivative		91.03 %	102.45 %	94.52 %	93.02 %	100.88 %
Mean aphid count	63.6250	57.9167	59.3333	56.0833	52.1667	52.6250
Plant #	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
exp1 1	56	12	9	6	8	4
exp1 2	16	4	4	3	2	0
exp1 3	23	11	11	11	12	3
exp1 4	96	21	37	20	23	12
exp1 5	93	22	22	24	22	8
exp1 6	27	15	24	11	10	7
exp2 1	133	17	16	14	14	11
exp2 2	9	6	5	7	7	3
exp2 3	76	27	16	12	12	6
exp2 4	11	5	7	6	6	2
exp2 5	121	33	17	8	7	7
exp2 6	39	10	13	11	8	4
exp3 1	78	27	15	13	6	4
exp3 2	99	34	19	18	24	9
exp3 3	67	22	11	10	12	4
exp3 4	132	29	28	18	23	8
exp3 5	120	44	26	10	11	3
exp3 6	48	22	22	5	3	3
exp4 1	57	14	12	10	6	5
exp4 2	72	23	14	12	11	6
exp4 3	74	29	25	18	7	6
exp4 4	27	19	15	21	13	3
exp4 5	18	7	5	2	5	1
exp4 6	45	21	12	14	12	5
Experimental survival rate	100.00 %	30.84 %	25.05 %	18.48 %	17.18 %	8.07 %
Survival rate derivative		30.84 %	81.22 %	73.77 %	92.96 %	46.97 %
Mean aphid count	64.0417	19.7500	16.0417	11.8333	11.0000	5.1667

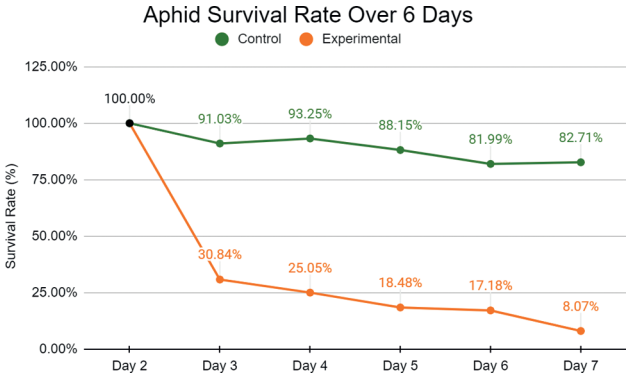

Fig. 3. Survival rate of experimental and control groups over 6 days.

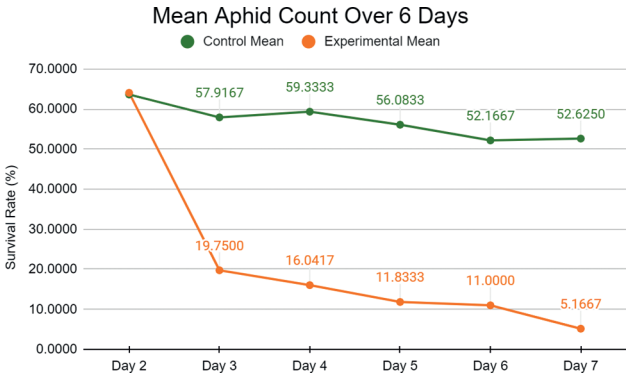

Fig. 4. Mean aphid count of experimental and control groups over 6 days.

overall decrease in the aphid population in the control group, although with increases between days 3 and 4 and between days 6 and 7.

A comparison of the mean aphid count of the two groups was visually documented in Fig. 4. By comparing the mean aphid count of the experimental and control groups on day 2 (63.625 vs 64.0417) as found in Tab. 3, it can be concluded that the experiment was successfully controlled due to similarity in aphid colonies between groups.

A comparison of the rate of change of survival rate of the two groups was visually documented in Fig. 5. The control derivative displays fairly stable consistency throughout the 6 days, which demonstrates little variation in the rate of change of the mean count and indicates an effectively controlled environment. The experimental derivative is less consistent due to the higher rate of change in the mean count, showing that the survival rate is more dramatic with the experimental group than in the control group.

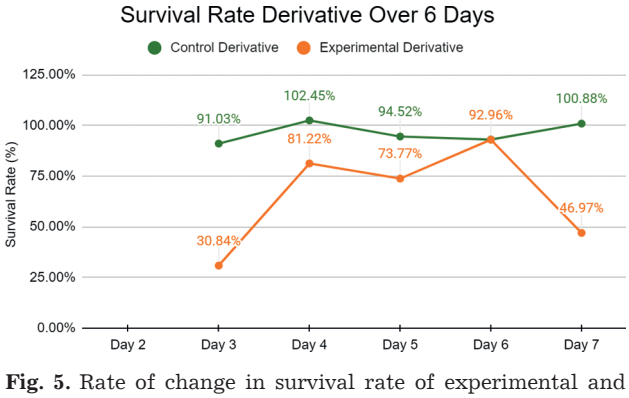

Fig. 5. Rate of change in survival rate of experimental and control groups over 6 days.

A T-Test was conducted to determine if *C. militaris* had a statistically significant effect on *B. brassicae* survival. The p-value on the second day of the experiment was 0.979262, but from the second day onwards, all p-values were less than 0.05, indicating that *C. militaris* exhibited a significant effect on *B. brassicae* survival from that day onwards (Tab. 4). In order to establish the size of this effect, Cohen's D effect sizes were calculated for all six days to produce a Z-score. On the second day, the effect size was 0.007512, indicating a small effect, but the effect sizes on all following days were less than –0.7, indicating a large effect on *B. brassicae* survival. It should be noted that on the final day, a Z-score of –1.120909 was produced.

The estimated corrected mortality (also referred to as inhibition of emergence, calculated using Abbott's correction formula) of *C. militaris* at the end of the experiment was 90.25 %, indicating that the pesticide satisfies the "effective" and "highly effective" criteria (roughly 70–95 % and 90–100 %, respectively) under the current pesticide registration system. (Sudo et al. 2019).

Tab. 4. Statistics calculated from the data in the aphid survival rate table.

	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
p-values	0.979262	0.008361	0.004473	0.003203	0.003811	0.000943
Z-scores	0.007512	–0.857054	–0.946100	–0.992477	–0.959674	–1.120909
Corrected Mortality	0.00 %	66.12 %	73.14 %	79.04 %	79.05 %	90.25 %

Discussion

In conclusion, the consistently lower survival rate of aphids sprayed by *C. militaris* and tap water compared to tap water alone indicates that *C. militaris* functions as an effective pest control for *B. brassicae*. It was observed that the aphid population increased in the control group between certain days, but it is uncertain as to why this is the case. The most likely reason to explain this is that a lack of proper population control allowed the aphid population to feed on the plants' nutrients and rebound in size. This was not observed in the experimental group likely because *C. militaris'* insecticidal properties allowed residue from the *C. militaris* spray to act as a physical barrier blocking aphids from properly feeding on the plants. Furthermore, mycelium was not observed on dead aphids, so it can be concluded that the aphids were either killed via chemical means or the aphids were killed by *C. militaris* spores but were not large enough for the fungus to grow off of their nutrients.

In order to enhance the thoroughness of this study, post-experimental documentation was conducted on plant health and aphid colony growth. Pictures of post-experimental observations in 2, 4 and 8 week intervals are displayed below in Fig. 6.

Although *C. militaris* with water no longer continued to be sprayed on the experimental group, inhibition of the aphid colony growth persisted in the experimental group while the control group experienced increased colony growth. This caused the new growth of the plants within the experimental group to be much more established and experience inhibited aphid colony growth in comparison to the control group. Eventually, after 5 weeks, aphid colony growth started once again on the experimental group. After 10 weeks, all the plants in the control group died, and after 14 weeks, all the plants in the experimental group died. Despite being initially disadvantaged by having less established plants, the experimental group was able to restore its health and outlive the control group, most likely as a result of *C. militaris'* insecticidal properties.

A concern identified during the experiment was the health of the experimental plants. The control plants grew much more than the experimental plants did during the experiment, and some plants experienced a decline in growth. This most likely was not caused by aphids, as their population was reduced on the experimental plants during the experiment. As such, the most likely reason for less growth on the experimental plants was that spraying large volumes of *C. militaris* created a film covering the surface of *B. oleracea* leaves, potentially inhibiting photosynthesis and thus, preventing plant growth. Spraying water, in quantities less than or equal to the volume of water sprayed on the control group during the experiment, after the application of *C. militaris* to plants will likely mitigate this problem. Moreover, using lower concentrations of *C. militaris* may exhibit similar aphid control effectiveness while creating less of a film and using fewer resources (highlighted in Appendix I). Lastly, the *C. militaris* spray may not need to be ap-

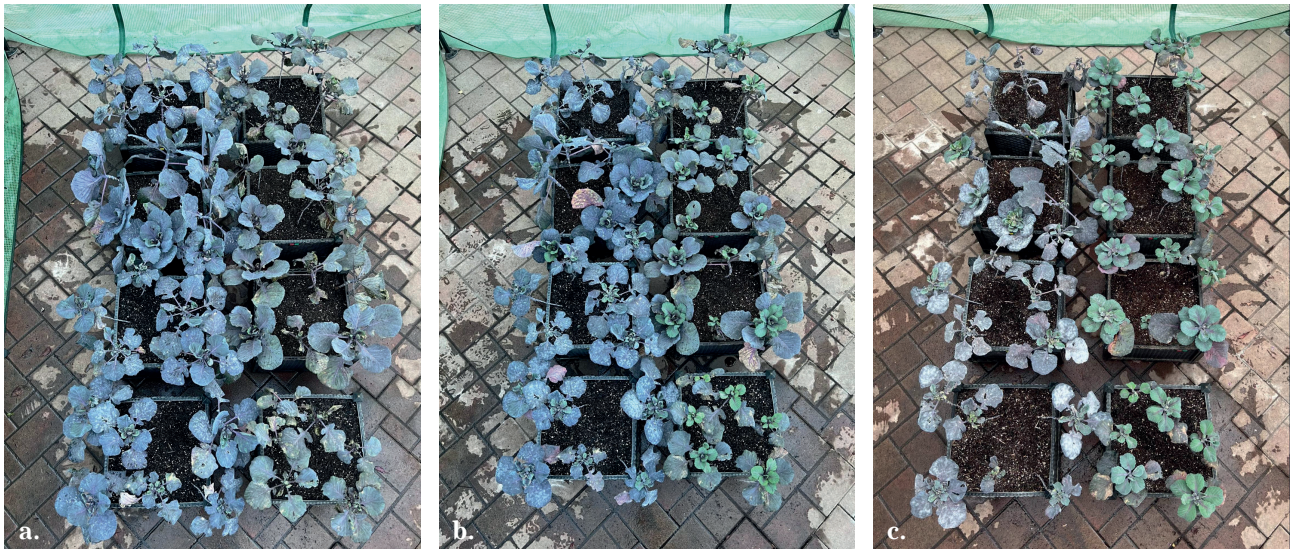

Fig. 6. Post-experimental observations of control and experimental groups (in each part figure the left column is the control group, and the right the experimental group). **a.** 2 weeks later; **b.** 4 weeks later; **c.** 8 weeks later.

plied as often to be equally as effective. This is suggested by the high initial decrease in survival rate and gradually lower changes in survival rate (indicated by the increasing survival rate derivatives of the experimental group) in the experimental group on the following days of the experiment (with the exception of the last day). Therefore, the effectiveness of *C. militaris* gradually decreased over time. Because there may have been extra amounts of *C. militaris* that did not exhibit an effect on aphid survival, *C. militaris* was likely sprayed more times than was needed to be effective. Thus, using lower concentrations and decreasing the number of applications may not have a large effect on survival rate, but will use fewer resources and decrease inhibition of photosynthesis on plants (an analysis of this is presented in Appendix I).

Another concern of this study regards limitations in terms of maximizing control during the experiment, and mainly involved the uneven light level distribution between experimental and control groups throughout each day. The surrounding environment presented shade at different times of the day, and the experimental and control groups were positioned such that each group received as equal amounts of light as possible throughout the day. However, exposure to different levels of light at different times prevented the experiment from being fully controlled. In the future, it may be beneficial to conduct this experiment in an open environment to prevent surrounding objects from creating shade.

Future works in the area of study (*B. brassicae* and the *Aphididae* family as a whole) should be conducted to confirm *C. militaris*' insecticidal properties since this experiment is the only known study conducted on *B. brassicae*. Furthermore, while this experiment served to expand the range of known targets of *C. militaris* beyond certain species of moths and ants, more experiments may expand this range even further (to common agricultural pests such as beetles, locusts, crickets, worms).

Next, it is important to address the expense of the potential application for this biopesticide. By adhering to the recommended instructions for *B. oleracea* plants in addition to usage limits based on health concerns set by the US Government, it was possible to calculate the cost of applying various commercial pesticides versus *C. militaris*, as demonstrated in Appendix I. It was assumed that a gallon of spray would be used for all pesticide applications, meaning that a corresponding amount of insecticide would be diluted with a certain amount of water. The cost of water ($1.50 per 1000 gallons) was negligible enough such that it was not factored into these calculations.

Currently, the cost to utilize *C. militaris* as used in this experiment (at a cost of $7.44/gal) is far too high for it to be practical in an agricultural setting, but following the analysis in Appendix I, a more optimized application of *C. militaris* (at a cost of $0.70/gal) will be much more feasible in an agricultural setting. Future studies can reduce the amount of *C. militaris* spray used and experiment with different concentrations and application intervals to find the most resource-efficient usage of *C. militaris*. Additional cost reductions may be possible through producing *C. militaris* from spawn and substrate rather than sourcing *C. militaris* from online retailers. For the sale of a *C. militaris*-based pesticide, the former will be more applicable and affordable. The full analysis of the cost to produce *C. militaris* is presented in Appendix II, resulting in a cost of $0.80 to produce one pound of *C. militaris*. Thus, the final application model is as follows: if all cost reductions, but no usage reductions, are taken into account (i.e. the current application method of 337.56 g of *C. militaris* per gallon of spray), the cost per gallon to apply *C. militaris* is $0.60; if all cost and usage reductions are taken into account (i.e. the optimized application method), the cost per gallon to apply *C. militaris* is $0.06. Putting this into perspective of other agricultural pesticides, selling *C. militaris* at lower prices for pesticide use will significantly drive down production costs and will make it a viable, safe alternative to existing agricultural pesticides.

Finally, to address real-world applications from this research, *C. militaris* may be used in an agricultural setting as a liquid concentrate or as solid pellets, both of which are then dissolved and diluted in water to maximize pesticide volume while maintaining toxicity. The resulting spray may be applied on the ground or in the air without any increase to cost because existing equipment can be used. *C. militaris* may also be combined with other ingredients to complement its potency and improve administration to pests. For instance, psilocybin-producing mushrooms, more commonly known as magic mushrooms, produce the alkaloid psilocybin to repel the feeding of insects (Wink 2003). Combining this with *C. militaris* may allow for a more defensive rather than an offensive pesticide, warding off most pests and killing the ones that remain. Alternatively, *Amanita muscaria* contains the compound muscimol, which activates inhibitory GABA neurotransmitters, producing sedative effects that attract insects toward itself, allowing the mushroom to act

as a toxic fly trap (McGonigle & Lummis 2010). The combination may result in an extremely destructive offensive pesticide in which pests are attracted to the pesticide and subsequently killed by *C. militaris*.

It may potentially be advantageous to utilize other mushroom species for insecticide use in addition *C. militaris* due to their lower cost of production; for instance, *Pleurotus ostreatus*, commonly known as oyster mushrooms, release the nerve gas 3-octanone to attack nematodes. As such, testing carnivorous mushrooms, like *P. ostreatus*, on other insects in a fashion similar to this study may uncover a cost-effective mycopesticide that can attack different ranges of insects than the range targeted by *C. militaris*, and combining these mycopesticides can yield one product to target a multitude of insect families.

No matter the recipe of the future application, a safe, optimized pesticide using *C. militaris* as the main active ingredient may effectively prevent pest infestation, thus allowing the agricultural industry to preserve food supplies without putting human lives at risk of injury or death and home gardeners to minimize health risks while effectively deterring pests from their plants.

Acknowledgements

The experimentation process would not have been possible without Ken Litchfield, who provided us with invaluable advice, ideas, knowledge, and guidance throughout the preparation of this study. His experience in the field of fungi, horticulture, and agriculture allowed us to properly design a scientific experiment and better cultivate all necessary living components. I would also like to thank Prof. Chris Brownell for providing in-depth information regarding statistical analysis methods to reinforce the results of this experiment. His extensive knowledge in statistics has been helpful with learning these new topics.

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(Manuscript accepted 24 May 2024; Corresponding Editor: I. Krisai-Greilhuber)

Appendix I: Calculating cost of C. militaris spray usage

Note that the first *C. militaris* entry is based upon its usage in this experiment. This was calculated by converting the concentration of the *C. militaris* spray (89.184g/L) to g/gal, which was 337.56g/gal. The cost to source *C. militaris* is at most $10/lb (when bought wholesale and in bulk, the price of fresh *C. militaris* fruiting bodies drastically decreases), meaning that a gallon of *C. militaris* spray using 337.56 g of *C. militaris* costs $7.44.

For a reduced usage of *C. militaris*, it was determined that 80 sprays of a solution when applied using the spray bottles in this experiment would expel 2 oz of that solution. Thus, applying four sprays (when applied as a fine mist) on the four corners of the top of the cabbage plant, four sprays on the four corners of the underside of the cabbage plant, two final sprays targeted at the growing tip of the plant, and doubling this amount to ensure full coverage would yield 20 total sprays adequate to cover the full surface area of the plant, meaning that 0.5 oz of solution may need to be sprayed per plant. The experimental usage was 2.667 oz of solution per plant, meaning that the proposed revised usage is 9.375 % of the previous usage (31.65g/gal). Multiplying this by the application cost of the previous usage results in a revised cost of $0.70 to apply one gallon of spray as opposed to $7.44.

Tab. 5. Cost to apply *C. militaris* versus other commercial insecticides.

Insecticide Name	Product Name	Cost of Application (Recommended by Manufacturer) Per Gallon of Water
Chlorpyrifos	Chlorpyrifos 4E AG	$10.60
C. militaris	—	$7.44
Deltamethrin	Bayer Polyzone Suspend	$5.18
Cypermethrin	Demon Max	$3.31
Permethrin	Perm-Up	$1.55
Gamma-Cyhalothrin	Spectracide Triazicide	$0.80
Revised *C. militaris*	—	**$0.70**
Bifenthrin	FMC Talstar Pro	$0.65
Malathion	Ortho MAX	$0.20

Appendix II: Cost to produce C. militaris

Mushrooms typically need to grow from a substrate consisting of a starch source and additional nutrients, depending on the species. The following recipe highlighted by William Padilla Brown's *The Cordyceps Cultivation Handbook* describes an optimal substrate for growing *C. militaris*:

- 1 gallon of water
- ¼ cup nutritional of yeast
- 2 tbsp of sugar
- 2 tbsp of azomite
- 72 pint jars (2 tbsp brown rice in each)

The cost to source each of these ingredients in bulk is listed in the table below. Bulk costs were acquired from wholesale products available to be bought in bulk online.

Thus, preparing 144 tbsp of brown rice substrate will cost $0.67. Translating this into the amount of *C. militaris* grown involves a concept called biological efficiency, which essentially measures how much biomass an organism produces given an amount of substrate. Biological efficiency is calculated by dividing the organism's biomass by the mass of dry substrate. In this way, it is possible to reach over 100 % biological efficiency due to the mass of wet substrate not being factored into the equation. For the purposes of this study, it can be assumed that the growing of *C. militaris* involves 100 % biological efficiency, meaning the mass of 144 tbsp of brown rice will completely translate into *C. militaris* biomass. Thus, scaling 144 tbsp of brown rice to a pound of brown rice (to keep measurements in line with the $10/lb figure), the cost to produce 1 pound of *C. militaris* is $0.80 when all ingredients are bought in bulk.

Tab. 6. Cost to source ingredients for *C. militaris* production.

Ingredient	**Cost to Purchase in Bulk**
1 gal water	$0.00
¼ cup nutritional yeast	$0.24
2 tbsp sugar	$0.03
2 tbsp azomite	$0.04
144 tbsp brown rice	$0.35
Total cost of 144tbsp substrate	$0.67