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ORIGINAL RESEARCH ARTICLE



Efficacy of bio agents and fungicides against *Colletotrichum* blight of large cardamom (*Amomum subulatum* Roxb.) under field condition in Sankhuwasabha District, Nepal

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ABSTRACT

The field experiment was carried out in five years old Golsai variety of large cardamom (*Amomum subulatum* Roxb.) at Khandbari-4, Pangma of Sankhuwasabha district between February and April 2022 to evaluate the efficacy of bioagents and fungicides against *Colletotrichum* blight of large cardamom (*A. subulatum*). Two bioagents, three fungicides, and control/water were taken as treatments, and the experiment was established in a Randomized Complete Block Design (RCBD) with an area of 4×3 sqm in each experimental unit having 6 treatments with 4 replications. Treatment wise application of bioagents and fungicides was given by using a high-volume knapsack sprayer with the required concentration. Subsequent sprays were given at seven-day intervals. The result of the study showed that among the tested fungicides, TOPCARE (Azoxystrobin 50% WDG) and bioagent *Trichoderma viride* significantly reduced blight under field conditions. So, spraying of the fungicide TOPCARE (Azoxystrobin 50% WDG) and bioagent *T. viride* will be best for controlling *Colletotrichum* blight on large cardamom in the Sankhuwasabha district.

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INTRODUCTION

The large cardamom i.e., *Amomum subulatum* Roxburgh is one of Nepal's commercially cultivated spices and cash crop; it belongs to Zingiberaceae family under the order Scitaminae and is a perennial herbaceous plant that grows wild in the Himalayas, throughout South-east Asia to northern Australia, and extends up to the Central Pacific (Yadav *et al.*, 2021; Joshi *et al.*, 2013). The Nepali name of the large cardamom is "Alainchi" and it is also popularly known as "Black Gold" as well as "Queen of Spices" and is sciophytic, tall, evergreen, monocot plant that is generally pollinated by bumblebees and grows well under the Uttish tree in the Eastern Himalayan part of Nepal (Yadav *et al.*, 2021). It was originated in Sikkim and Roxburgh first elaborated on this plant in his 'Plants of the Coast of Coromandel' and in

'Flora Indica' (Bhattarai *et al.*, 2013). It is cultivated at an altitude of 500–1800 m on slopes under diversified shade, preferably along streams, and grows well in mid hills receiving high rainfall, sandy loam soil with an acidic pH, rich in organic matter, and humid climate (Rout *et al.*, 2003; Joshi *et al.*, 2013). It has short-stalked spike-bearing bisexual allogamous flowers, ripe fruit contains seed capsules, and the fruit is tri locular, reddish brown, and contains a dark pink seed capsule (Kishore *et al.*, 2011; Shrestha *et al.*, 2018).

Large cardamom (*Amomum subulatum* Roxburgh) is possibly the oldest spice crop known to humans. It is widely used for Ayurvedic preparations and has a high market value due to the capsule, i.e., the seed has its high economic importance (Gudade *et al.*, 2013; Belbase *et al.*, 2018). The seeds of large cardamom are used as a precautionary as wells as therapeutic agent for

throat problems, congestion of the lungs, eyelids tenderness, digestive disorders, and the treatment of pulmonary tuberculosis (Pathak, 2008). The fruits of large cardamom are generally known as "Heel Kalan" or "Bari Ilaichi" and are mostly prescribed for the treatment of gastrointestinal disorders in the Unani system of medicine (Jafri et al., 2001). It contains 2-3 % essential oil, which is used as a flavoring agent in many industries, including food and beverages, confectionery, and cosmetics, and is also used to treat migraines (Pathak, 2008; Joshi et al., 2013). It was believed that the Lepchas were the first to collect large cardamom capsules from natural forests, mainly for the medicinal purposes and also as an aromatic edible wild fruit (Gudade et al., 2013).

Large cardamom was first cultivated in the Ilam district in 1865; commercial cultivation began in late 1950; and recently, large cardamom is being grown commercially in 51 districts of Nepal, primarily in the eastern hill and mountain areas and expanding to the western parts. In total, 21,960 households from 51 districts are engaged in cardamom cultivation in Nepal, with Taplejung being the largest cardamom producing district, followed by Panchthar, Ilam, and Sankhuwasabha (Shrestha et al., 2018; Belbase et al., 2018). Nepal produces about 9,545 metric tons of large cardamom from an area of 18,748 hectares with a 0.58 mt/ha yield (Karki and K.C., 2020 and 2021). 14 varieties of large cardamom are being cultivated, and recently, the Nepalese government registered 5 different varieties of large cardamom, namely, Ramsai, Golsai, Dambarsai, Jirmale and Bharlyange (Yadav et al., 2021). The biggest risk to growing cardamom, which results in a loss in productivity and output, is disease. Numerous diseases have caused the quality and yield of the fruits to decline. The prevalence of the disease varies depending on the prevailing pathogen population and climatic conditions. The choice of suitable cultivars based on the current climatic conditions and the presence of diseases, however, is unknown to farmers. They lack the knowledge necessary to implement effective procedures for managing diseases and cultivating crops. It will be useful to understand the prevalence and severity of the disease associated with blight through the study. Aphids are responsible for transmitting viral diseases, such as Chirkey and Foorkey, while Colletotrichum blight has emerged as the major diseases of large cardamom, causing heavy losses and a decline in plant populations (Vijayan et al., 2014). It is a disastrous, widespread, and economically important disease of large cardamom that leads to an annual yield loss of about 7-13% and was first identified in the Kerela state of India (Manju et al., 2018). Symptoms appear as blackish-brown lesions at first in the pseudo stem, which gradually change to grey or black patches with brown margins. Most often, these lesions become necrotic, resulting in the drying of whole leaves and giving them a burnt-up appearance. In severe cases, the pseudo stem breaks at the site of the necrotic lesion, causing the entire plant to dry up (Saju et al., 2013). The effectiveness of several bioagents and fungicides in managing leaf blight in big cardamom is supported by this study. The production of huge cardamom can be significantly increased by reducing blight. Blight has acquired fungicid-

al resistance as a result of excessive and inappropriate pesticide use. It is crucial to understand how compounds can generate fungicidal toxicity. So, the proper use of bioagents and fungicides that prioritize the growth of healthy crops, an increase in yield, and the maintenance of a sustainable agroecosystem can result in effective management of leaf blight. Considering the economic importance of the crop, present exploration was undertaken to evaluate the efficacy of bioagents, i.e., *Pseudomonas fluorescens* and *Trichoderma viride* and fungicides, i.e., TOPCARE (Azoxystrobin 50% WDG), DHANUKA M-45 (Mancozeb 75% WP), and NAGCOPER (Copper Oxychloride 50% WP) against Colletotrichum blight of large cardamom.

MATERIALS AND METHODS

Site selection

This experiment was conducted at a large cardamom field located at Pangma-4, Khandbari, Sankhuwasabha district, the eastern part of the temperate region of Nepal. The experiment site was located at an elevation of 1150 m. Geographically, it was located at a latitude of 27°24'41" N and a longitude of 87°11'14" E. The experiment was carried out from February 8 to April 15, 2022.

Field preparation

The old, damaged, and diseased parts of large cardamom were manually and mechanically removed and burned away from the field to prevent perennating pathogens from infecting the plant.

Experimental design

The experiment was established in a Randomized Complete Block Design (RCBD) with an area of 4x3 sqm in each experimental unit having 6 treatments with 4 replications.

Treatment details

T₁= TOPCARE (Azoxystrobin 50% WDG) 0.1%
 T₂= DHANUKA M-45 (Mancozeb 75%WP) 0.225%
 T₃= *Trichoderma viride* 0.5%
 T₄= *Pseudomonas fluorescens* 0.5%
 T₅= NAGCOPER (Copper oxy Chloride 50%WP)
 T₆= Control/water

Allocation and spraying of bioagents and fungicides

Different treatments were assigned to the different experimental units in each replication by the lottery method. The treatment consists of various bioagents and fungicides. The bioagents and fungicides were applied on a weekly basis. Treatment wise application of bioagents and fungicides was given by using a high-volume knapsack sprayer with the required concentration. The subsequent spray was given at a seven days interval.

Observation

The observation of leaf blight, i.e., *Colletotrichum gloeosporioides*, on the basis of disease incidence and percentage disease index was recorded from three randomly selected plants per treatment per replication before spray and seven days after each

Table 1. Disease scoring was done using 0-5 scales (Kadam et al., 2014).

Scale	Percentage leaf infection
0	No symptoms
1	(1-10) % symptoms on leaf
2	(11-20) % symptoms on leaf
3	(20-30) % symptoms on leaf
4	(30-40) % symptoms on leaf
5	>40 % symptoms on leaf

spray. On March 13, 2022, the first spray was applied. The plants were sprayed three times at seven-day intervals during the research period.

Percentage Disease Index (PDI) was calculated by following Mahapatra and Das (2013).

$$PDI = \frac{\sum \text{of rating of infected leaves on plant}}{\text{No. of leaves observed} \times \text{maximum disease score}} \times 100$$

$$AUDPC = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

AUDPC = Area Under Disease Progression Curve

y_i = assessment of diseases at i^{th} observation

t_i = time (in days, hours, etc.) at i^{th} observation

n = total no. of observations

$$DI (\%) = \frac{\text{no. of infected plants}}{\text{total no. of plants}} \times 100$$

Statistical analysis

The experimental data were recorded in Microsoft Excel and analyzed statistically by using Gene-stat for Analysis of Variance (ANOVA), and a mean comparison was done following the Duncan Multiple Range Test (DMRT) at a 5% level of probability.

RESULTS AND DISCUSSION

PDI on efficacy of different bio agents and fungicides against Colletotrichum blight (C. gloeosporioides) of large cardamom

The effects of treatments on severity of Colletotrichum blight, in terms of PDI was presented in Table 2. which showed that before spraying of bio agents and fungicides, the PDI of TOPCARE was recorded 69.05a followed by DHANUKA M-45 74.30ab, NAGCOPER 75.30ab, *Trichoderma viride* 82.55b, control 84.50b and *Pseudomonas fluorescens* 84.70b with CV (%)

10.2, LSD_{0.05} 12.06, Sem \pm 4 and F-value 0.471 resulting non-significant test at 5 % level of significance. At 7th day of 1st spray, the lowest PDI was observed in plot treated with TOPCARE i.e., 44.70a followed by DHANUKA M-45 i.e., 51.05ab. The PDI of plot treated with NAGCOPER was 57.35bc and was statistically similar with the plot treated with *T. viride* i.e., 61.00bc. The PDI of plots treated with *P. fluorescens* was 64.45c and was statistically similar to the plot with control which had highest PDI. Among 5 treatments, TOPCARE was most effective fungicides followed by DHANUKA M-45, NAGCOPER and bioagent *T. viride*. At 7th day of 2nd spray, the lowest PDI was observed in plot treated with TOPCARE i.e., 37.45a followed by DHANUKA M-45 i.e., 49.10ab. The PDI of plot treated with NAGCOPER was 56.10bc and was statistically similar with the plot treated with *T. viride* i.e., 59.45bc. The PDI of plots treated with *P. fluorescens* was 62.75c and was statistically similar to the plot with control which had highest PDI. Among 5 treatments, TOPCARE was most effective fungicides followed by DHANUKA M-45, NAGCOPER and bioagent *T. viride*.

At 7th day of 3rd spray, the lowest PDI was observed in plot treated with TOPCARE i.e., 28.95a followed by DHANUKA M-45 i.e., 46.00b which was statistically similar to the plot treated with NAGCOPER 53.80b. The PDI of plot treated with *T. viride* i.e., 56.00bc and was statistically similar to the plot treated with *P. fluorescens* is 60.65bc. The plot with control which had highest PDI i.e., 69.00c. Among 5 treatments, TOPCARE was most effective fungicides followed by DHANUKA M-45, NAGCOPER and bioagent *T. viride* and *P. fluorescens*. Similarly, at 7th day of 1st spray, decrease of PDI over control was recorded 32.32% in TOPCARE followed by 22.59% in DHANUKA M-45, 13.04% in NAGCOPER, 7.51% in *T. viride* and 2.27% in *P. fluorescens*. The highest disease control percentage was recorded in TOPCARE. At 7th day of 2nd spray, decrease of PDI over control was recorded 44.06% in TOPCARE followed by 26.66% in DHANUKA M-45, 16.2% in NAGCOPER, 11.2% in *T. viride* and 6.27% in *P. fluorescens*. The highest disease control percentage was recorded in TOPCARE. At 7th day of 3rd spray, decrease of PDI over control was recorded 58.04% in TOPCARE followed by 33.33% in DHANUKA M-45, 22.03% in NAGCOPER, 18.8% in *T. viride*. and 12.1% in *P. fluorescens*. The highest disease control percentage was recorded in TOPCARE.

Table 2. PDI on efficacy of different bioagents and fungicides against Colletotrichum blight.

Treatments	Before spray PDI	After 1 st spray		After 2 nd spray		After 3 rd spray	
		PDI	Decrease of PDI over control (%)	PDI	Decrease of PDI over control (%)	PDI	Decrease of PDI over control (%)
TOPCARE (0.1%)	69.05a	44.70a	32.32	37.45a	44.06	28.95a	58.04
DHANUKA M-45 (0.225%)	74.30ab	51.05ab	22.59	49.10ab	26.66	46.00b	33.33
<i>T. viride</i> (0.5%)	82.55b	61.00c	7.51	59.45bc	11.2	56.00c	18.8
<i>P. fluorescens</i> (0.5%)	84.70b	64.45c	2.27	62.75c	6.27	60.65bc	12.1
NAGCOPER (0.125%)	75.30ab	57.35bc	13.04	56.10bc	16.2	53.80b	22.03
Control/water	84.50b	65.95c	-	66.95c	-	69.00c	-
Sem \pm	4	3.96		4.08		4.57	
CV (%)	10.2	13.8		14.8		17.4	
LSD	12.06	11.95		12.30		13.77	
F-test	NS	*		*		*	

CV: Coefficient of Variation; *: significant at 5% level of significance, LSD: Least Significant Difference; Sem: Standard error of means. Collected data were analyzed statistically by using Gene-stat and mean comparison was done following Duncan Multiple Range Test (DMRT) at 5% level of probability.

Table 3. Colletotrichum blight AUDPC score on large cardamom in Sankhuwasabha, Nepal.

Treatments	AUDPC score
TOPCARE (0.1%)	918a
DHANUKA M-45 (0.225%)	1122ab
<i>T. viride</i> (0.5%)	1313bc
<i>P. fluorescens</i> (0.5%)	1399c
NAGCOPER (0.125%)	1246bc
Control/water	1468c
Grand mean	1244
Sem±	83.8
CV (%)	13.5
LSD	252.6
F-test	*

CV: Coefficient of Variation; *: significant at 5% level of significance, LSD: Least Significant Difference; Sem: Standard error of means. Collected data were analyzed statistically by using Gene-stat and mean comparison was done following Duncan Multiple Range Test (DMRT) at 5% and 1% level of probability.

Table 4. Disease incidence percentage (DI%) of *C. gloeosporioides* causing blight on large cardamom.

Treatments	DI% before spray	DI% after 3 rd spray
TOPCARE (0.1%)	70.09a	20.09a
DHANUKA M-45 (0.225%)	70.09a	36.60b
<i>T. viride</i> (0.5%)	59.43a	37.35b
<i>P. fluorescens</i> (0.5%)	62.50a	54.17c
NAGCOPER (0.125%)	64.88a	30.31ab
Control/water	62.50a	84.38d
Grand mean	64.9	43.8
Sem±	4.45	3.46
LSD	13.42	10.44
CV%	13.7	15.8
F test	NS	**

CV: Coefficient of Variation; *: significant at 5% level of significance, LSD: Least Significant Difference; Sem: Standard error of means. Collected data were analyzed statistically by using Gene-stat and mean comparison was done following Duncan Multiple Range Test (DMRT) at 5% and 1% level of probability.

Efficacy of different bioagents and fungicides against Colletotrichum blight (*C. gloeosporioides*) on AUDPC score of large cardamom

The efficacy of bioagents and fungicides against Colletotrichum blight on AUDPC score of large cardamom is presented in table 3. Among the treatments used in research, the AUDPC score of TOPCARE (918a) was observed statistically significant over DHANUKA M-45 (1122ab), NAGCOPER (1246bc), *T. viride* (1313bc), *P. fluorescens* (1399c), and control (1468c). The AUDPC score of *P. fluorescens* is 1399c was statistically similar with control.

Efficacy of different bioagents and fungicides against Colletotrichum blight (*C. gloeosporioides*) on DI (%) of large cardamom

The efficacy of bioagents and fungicides against Colletotrichum blight in terms of DI% of large cardamom is presented in table 4. Before spraying bio agents and fungicides, the Disease Incidence percentage (DI%) was observed highest in plots to be treated with TOPCARE and DHANUKA M-45 i.e., 70.09a and was statistically similar with the plots treated by NAGCOPER i.e., 64.88a, *P. fluorescens* 62.50a and control i.e., 62.50a and *T. viride* i.e., 59.43a. After application of bio agents and fungicides, the Disease Incidence percentage (DI%) was observed lowest in plots treated with TOPCARE i.e., 20.09a followed by NAGCOPER i.e., 30.31ab. The DI% of DHANUKA M-45 i.e., 36.60b and

was statistically similar with DI% of *T. viride* i.e., 37.35b. The DI% of *P. fluorescens* i.e., 54.17c and control 84.38d.

In the research, it was found that TOPCARE (Azoxystrobin 50% WDG) reduced greatly Colletotrichum blight of large cardamom followed by DHANUKA M-45, NAGCOPER, *T. viride* and *P. fluorescens*. *T. viride* showed greater efficacy than *P. fluorescens*. Study showed that control treatment where we sprayed water was seen ineffective which is due to climatic and environmental factors. The development of Colletotrichum blight disease highly depend upon the environmental parameters. TOPCARE had been reported effective against management of Colletotrichum blight. The findings are in accordance with the findings of Gusain and Ravi (2020) who found the efficacy of blight disease is managed by mancozeb, copper oxychloride, *T. viride* and *P. fluorescens*. TOPCARE (Azoxystrobin 50% WDG) is a broad-spectrum systemic fungicide belonging to strobilurins family which is developed from naturally occurring antifungal compound found in mushroom fungus present on decayed wood which inhibits the mycelial growth of *C. gloeosporioides* resulting control of disease (Sundravadana et al., 2007). Azoxystrobin causes various crucial phases of fungal development to be disrupted or to cause mycelial collapse (Sundravadana et al., 2006). *Trichoderma* have different hostile possibilities, and this variation may be exacerbated by the genetic background and isolation site, which inhibit the pathogen's growth through its rapid growth potential and competition for food and space, as well as inactivate the

enzymes produced by the pathogen and develop host resistance (Sarfray et al., 2018).

The AUDPC score of TOPCARE (Azoxystrobin 50% WDG) was low as compare to other fungicides and bio agents used while control had the highest AUDPC score. It showed that the most effective fungicides against Colletotrichum blight was TOPCARE (Azoxystrobin 50%WDG) which was in accordance with the findings of (Shetty et al., 2014), who found that the AUDPC score of experimental units treated with TOPCARE (Azoxystrobin 50% WDG) was significantly lower than plot treated with control. In an experiment, it was found that the DI % changed at different time intervals in research field as the efficacy of bioagents and fungicides against Colletotrichum blight was affected by several environmental parameters such as temperature, relative humidity and precipitation as well as the concentration of the fungicides which was in accordance with the findings of (Kadam, et al., 2014), who found that Colletotrichum blight of large cardamom had positive relation with maximum temperature, RH and precipitation and vice-versa. As a result, it was found that TOPCARE (Azoxystrobin 50% WDG) was the most effective fungicide among the tested fungicides and bio agents *T. viride* was effective as compared to *P. fluorescens*.

Conclusion

This study was conducted to find out the efficacy of bioagents and fungicides against pathogens. For the management of this disease, effective fungicides are required as they have fast acting mechanism as compared to the bioagents and botanicals. Proper and timely applications of effective fungicides check growth of the fungus on developing plants. So, chemical control is the most effective management for crop protection in limited time. From an experiment, it was concluded that TOPCARE (Azoxystrobin 50% WDG) followed by DHANUKA M-45 (Mancozeb 75% WP) were the effective ones among the tested fungicides and the bioagent *T. viride* can be alternative source for effectively controlling Colletotrichum blight under field conditions. The findings of this research will be fruitful in managing blight disease of large cardamom. Also, this research will be useful for student and researchers. There is need to develop a separate Spice Research Institute for large cardamom research and development in a multidisciplinary approach. The government and private sectors should incorporate research, and technology generation, and quality control at the farmer's level. Research institutions like NARC should develop appropriate, high-yielding varieties suitable for different bio physical domains in Nepal. Similarly, measures to overcome disease and pests should be developed. Further trials of various bioagents, such as essential oils, in infected plants should be conducted to determine their efficacy under field conditions. An awareness program should be conducted for the farmers to learn about the disease and pests. Disease-susceptible varieties should be discouraged from cultivation. Farmers should be provided with good farming skills, knowledge, and training programs regarding

the damage caused by the disease, its symptoms, and management strategies. Hence, considering the present situation of the experiment, this experiment should be carried out for at least 2 years for the confirmation of the results.

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