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Evaluation of Copper Fungicide in Anthracnose of Pumpkin under Organic Cultivation in Assam

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ABSTRACT: Anthracnose which is caused by *Colletotrichum lagenarium*, is a serious disease that can infect all the aerial parts and fruits of the pumpkin plant, resulting in significant crop loss. In this study, the effectiveness of three different forms of copper fungicides- Copper Oxychloride 50% WP, Copper Hydroxide and Bordeaux mixture were evaluated against anthracnose at 10, 25, 50, and 100 ppm concentrations. The poisoned food technique was adopted for the evaluation *in vitro*. The fungicide Copper Oxychloride 50% WP was found to be most effective in suppressing the mycelial growth by 72.10% at 100 ppm, compared to the control. Copper Oxychloride 50% WP was further evaluated in field conditions under six different spray schedules and was found effective in reducing the incidence and intensity of the disease to a varying extent. Application of 4 nos. of sprays at 10-day intervals could provide the highest protection of 77.80% against the disease with a maximum yield of 1.78 t/ha.

Keywords: Anthracnose, Copper fungicides, Organic cultivation, Disease intensity, Bordeaux mixture, Copper oxychloride, Mancozeb.

INTRODUCTION

Pumpkin (*Cucurbita moschata* Duch) belongs to the family Cucurbitaceae and is a widely cultivated crop throughout the year. It is a warm-season crop that thrives in well-drained, organic-rich soil. Pumpkins are cultivated throughout the world for several reasons including agriculture, commercial and decorative sales. The United States, Canada, Mexico, India, and China are the top five global producers of pumpkin. The world's total pumpkin production is around 28 million tonnes, with a yield of 0.93(“000 tonnes) per hectare, compared to India's production of 2030 metric tonnes (National Horticulture Board, 2020). The requirement for year-round pumpkin production has resulted in a significant increase in the number of pests and diseases that affect the crop. Among many challenges that arise during the production of pumpkins, insect pests and diseases rank first which results in crop production losses.

This crop is frequently affected by several foliar fungal diseases, resulting in significant loss of production. Diseases that affect pumpkins include: Downy mildew (*Pseudoperonospora cubensis*), Anthracnose (*Colletotrichum* spp.), Powdery mildew (*Sphaerotheca fuliginea*, *Erysiphe cichoracearum*), Fusarium wilt (*Fusarium* spp.), Cercospora leaf spot (*Cercospora citrullina*), Bacterial wilt (*Erwinia tracheiphila*), Phytophthora crown and root rot (*Phytophthora capsici*) inflict huge economic losses worldwide (Zitter *et al*., 1998; Saha, 2002). Among all diseases, anthracnose (*Colletotrichum lagenarium*) is a serious disease which infects all the aerial parts of the pumpkin plant and manifests as angular to roughly circular reddish-brown lesions on leaves. Under humid conditions, the lesions become dotted with pinkish masses of conidia. Conidia are mostly produced singly and occasionally in chains from acervuli acrogenously (Vashishta, 1999). This disease occurs at any time of the year, even though the majority of the damage happens late in the season after the fruit has developed. Anthracnose infection and spread are mostly facilitated by warm, humid conditions, with optimal temperatures falling between 26 to 32°C. In severe attacks, anthracnose may reduce crop yield. Such attacks occur mostly in the early season when conditions with sufficient precipitation and temperature of 32ºC prevail (Thompson and Jenkins 1985). The pathogen survives as conidia in plant debris and seeds (Palenchar *et al*., 2012).

Typically, growers apply fungicides such as Mancozeb to suppress the disease; if left unchecked, this might result in significant crop loss. However, as a result of the increasing demand for vegetables, even pumpkin has recently gained economic significance in Assam. As a result, vegetable growers are worried about controlling crop diseases, but they have few options when it comes to fungicide selection, with copper-based fungicides being one of the most effective because the crop is sensitive to this element. Some of the advantages of copper-based fungicides include their high efficiency against several fungal pathogens such as *Pseudoperonospora cubensis, Sphaerotheca fuliginea*, *Erysiphe cichoracearum*, *Colletotrichum* sp. and their reduced cost; their low toxicity for cucurbit plants and long persistence; the low potential for the appearance of resistant isolates due to the multisite mode of action of the Cu^{2+} ion; and their use is authorized, even after flowering, in both organic and conventional farming (Roca *et al*., 2007; Cacciola *et al*., 2012; Moral *et al*., 2018).

Assam is a state that produces organic crops by default. The different methods used for organic production technology are directly related to the quality of the produce. The market for organic produce is growing day by day. However, pumpkin production in Assam is extremely difficult due to the warm and humid weather conditions during the growing season, which encourages the rapid spread of anthracnose disease. The use of synthetic fungicides is prohibited in organic agriculture, except for copper products, which are allowed in restricted quantities to treat diseases that cannot be controlled by cultural methods alone. Copper fungicides are now included in the National Centre of Organic Farming, Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India's list of approved products. They are regulated for use as disease management agents, with a maximum application rate of 8 kg/hectare per year. The evaluation of fungicide efficacy under organic conditions allowed us to screen several antifungal effects before field trials. During these evaluations, in general, compounds must be tested by *in-vitro* assays against the pathogen's conidial germination and mycelial growth (Dhingra and Sinclair 1995). Likewise, the evaluation of copper fungicides to control plant disease by using field plants enables an initial screening (Knight *et al.,* 1997). The final goal of this study is to evaluate copper-based fungicides that require a minimum quantity of metallic copper to control anthracnose effectively.

MATERIALS AND METHODS

Study Area: The study was conducted during the *rabi* season of 2021 to 2022, in the ICR experimental Farm of AAU, Jorhat, under well-drained medium organic soil conditions. During the height of monsoon season, the field is temporarily free of water logging. It has a hot, humid subtropical season (May to August) and a comparatively cold, dry winter (November to February). The hottest month of the year is August. August's average temperature is 28.1°C. The coldest month is January, with average temperatures of 17.2°C.

***In-vitro* evaluation of copper fungicides in reducing mycelial growth of *Colletotrichum lagenarium*:** Three copper fungicides *viz.*, Copper Oxychloride 50% WP (TRUCOP 50% WP), Copper hydroxide 53.8 % w/w (KOCIDE, 2000), and Bordeaux mixture were tested *in vitro* for fungicidal activity against the pathogen. All the test fungicides were assessed for their efficiency against mycelial growth suppression using the poisoned food approach at various doses *viz.*, 10, 25, 50, and 100 ppm concentrations (Schmitz, 1930) in the mycology laboratory of the Department of Plant Pathology, AAU, Jorhat. Per cent mycelial inhibition was calculated as described by Vincent (1947):

Percent inhibition = C – T/C × 100

Where,

C = Growth of fungus in control (mm)

T = Growth of fungus in treatment (mm)

The most effective fungicide against the pathogen was selected for *in vivo* evaluation in field trial for controlling the anthracnose disease in pumpkin.

Treatment combinations for the investigation: A field trial experiment was conducted in a randomized block design (RBD) to evaluate the efficacies of the most efficient fungicide examined *in vitro* shown in Fig. 1.

Location	: ICR Farm, AAU, Jorhat
Period	: October 2021-May 2022
Variety	: Arjuna
Design	: Randomized Block Design (RBD)
Replications	: 3
Treatments	: 7
Plot size	: 7 m × 3 m
Total no. of plots	: 21

T_1: Application of 2 numbers of spray @ 0.2% with the most effective fungicide (selected from the *in vitro* study) at 10 days interval

T_2: Application of 3 numbers of spray @ 0.2% with the most effective fungicide (selected from the *in vitro* study) at 10 days interval

T_3: Application of 4 numbers of spray @ 0.2% with the most effective fungicide (selected from the *in vitro* study) at 10 days interval

T_4: Application of 2 numbers of spray @ 0.25% with the most effective fungicide (selected from the *in vitro* study) at 10 days interval

T_5: Application of 3 numbers of spray @ 0.25% with the most effective fungicide (selected from the *in vitro* study) at 10 days interval

T_6: Application of 4 numbers of spray @ 0.25% with the most effective fungicide (selected from the *in vitro* study) at 10 days interval

T_7: Absolute Control (Unsprayed)

Estimation of disease incidence and disease intensity

After emergence, plots were scouted for anthracnose symptoms caused by infection. As soon as symptoms were detected, all plots were visually assessed for anthracnose intensity (%) in leaves and fruits as described by Singh *et al.* (1996). The disease intensity was calculated as:

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total no. of ratings} \times \text{Maximum disease score}} \times 100$$

The disease intensity was assessed using 0-4 disease scale modified by Singh *et al.* (1996).

The scale used is

Disease score/ rating	Leaf area affected (%)
0	No disease
1	0.1-10%
2	10.1-20%
3	20.1-50%
4	More than 50 %

The following formula was used to compute disease incidence:

$$\text{Disease incidence (\%)} = \frac{\text{No.of infected plants per plot}}{\text{Total number of plants per plot}} \times 100$$

Statistical analysis. The acquired data were statistically analysed using Fisher's Analysis of Variance. The significance of variation among observed data was assessed by computing the "F" value and then comparing it to the tabular value of "F" at a 5% level of probability. An angular transformation was used to transform the percentage numbers. Further, the treatment means were compared among themselves by calculating critical difference (CD) by the following formula:

C.D. at 5 % = S.Ed × 't' 5 % (at error d.f)

The standard error of difference of mean (S.Ed) was calculated by the following formula:

$$\text{S.Ed} \pm = \sqrt{\frac{2 \times \text{error mean square}}{\text{Number of replications}}}$$

Where, S.Ed = Standard error of difference

't' 5 % = "t" for error d.f. at 5 % per cent level of probability.

The significance and non-significance of the treatments at 5 % level of probability were calculated out by multiplying the S.Ed with appropriate tabulated value for error degrees of freedom.

RESULTS AND DISCUSSIONS

The efficacy of three copper-based fungicides *viz.*, Copper Oxychloride 50% WP (TRUCOP 50% WP), Copper Hydroxide 53.8 % w/w (KOCIDE 2000) and Bordeaux mixture were studied at different concentrations of 10, 25, 50, and 100 ppm under *in-vitro* conditions through Poisoned Food Technique against *Colletotrichum lagenarium*. The data on the mycelial growth of *Colletotrichum lagenarium* was recorded for each fungicide at given concentrations and is presented in Table 1. *In-vitro* studies indicated that all the fungicides used for evaluation were effective against the pathogen *Colletotrichum lagenarium,* but Copper Oxychloride followed by Copper Hydroxide was found to be a highly effective fungicide treatment against *Colletotrichum lagenarium*. Since Copper Oxychloride recorded the highest mycelia growth inhibition against the pathogen *C. lagenarium* over two other copper fungicides *viz.,* Copper Hydroxide and Bordeaux mixture, it was selected for *in vivo* assessment of the management of anthracnose of pumpkin under field conditions. The results of this investigation are consistent with the findings of Gopinath *et al*. (2006); Kumar (2008); Gawade *et al*. (2009), who similarly reported that copper oxychloride was the most effective fungicide against *Colletotrichum lagenarium.* Roja and Kulkarni (2022) in their research on pod blight of soybean caused by *Colletotrichum truncatum,* discovered that all the combinations of copper fungicides used in the treatment were highly effective in completely inhibiting the growth of the pathogen's mycelia under *in vitro* condition, resulting in a high percentage of mycelial inhibition.

The best effective copper fungicide in *in-vitro* evaluation, Copper Oxychloride 50% WP was tested against anthracnose of pumpkin under different spray schedules under field conditions. The fungicide was sprayed at @0.2% and @0.25% concentrations under three separate spray schedules (2, 3 and 4 numbers of spray at 10-day intervals) starting from the first appearance of the anthracnose disease. The experimental results are presented in Table 2 and Fig. 2. The first appearance of anthracnose disease in all the treated and untreated control plots was recorded on the 10th of December 2021. The average per cent disease incidence ranging from 11.13 to 16.70 per cent was recorded in different treatments, which are found to be statistically not significant. An incidence of 16.70 per cent was recorded on the control plot (T_7) whereas, a minimum incidence of 11.13 per cent was recorded in the plots T_3, T_5 and T_6, respectively. However, on the same day, an incidence of 16.70 per cent was recorded in the plots T_1, T_2 and T_4 respectively. After 7 days of the first appearance of the disease, the lowest disease incidence of 16.70 per cent was recorded in the treatments T_4, T_5 and T_6 respectively. The highest disease incidence of 50.00 per cent was recorded in the control treatment T_7. In other treatments *viz.*, T_1, T_2 and T_3 recorded the disease incidence of 33.30 per cent, 27.77 per cent and 22.23 per cent, respectively. After 14 days, a minimum of 16.70 per cent incidence of anthracnose disease was recorded in T_6 followed by a disease incidence of 22.23 per cent in plot T_4. The treatment plots T_3 and T_5 recorded a disease of 27.77 per cent, respectively. On the same day, the treatment plots T_1 and T_2 recorded disease incidence of 38.87 per cent and 33.30 per cent, respectively. Meanwhile, the highest disease incidence of 72.23 per cent was recorded in the control plot T_7. After 21 days, the lowest disease incidence of 22.23 per cent was recorded in plot T_6 whereas, a disease incidence of 27.70 per cent was recorded in the T_5 plot. On the contrary, a maximum incidence of 88.87 per cent was recorded in the control plot T_7. Other treatments *viz.*, T_1, T_2, T_3 and T_4 recorded disease incidence of 50.00 per cent, 44.43 per cent, 38.87 per cent and 33.30 per cent of the disease incidence, respectively. After 35 days, a minimum disease incidence of 38.87 per cent was recorded in T_6, while a maximum incidence of 100.00 per cent was recorded in the control plot T_7. However, an incidence of 50.00 per cent was recorded in the T_5 plot. In contrast to that, an incidence of 61.13 per cent was recorded in the T_4 plot. The plots T_1, T_2 and T_3 recorded incidences of 77.77 per cent, 72.23 per cent and 66.70 per cent respectively.

Copper Oxychloride 50% WP was found to be effective in reducing the occurrence and severity of anthracnose. Ullasa *et al.* (1986) made a similar observation, suggesting that Copper Oxychloride can successfully control anthracnose if applied prophylactically to all plant surfaces, which validates the current study's findings. Copper Oxychloride's increased efficacy against anthracnose may be related to higher copper ion release and superior susceptibility. Copper fungicide efficiency can be greatly boosted by reducing the particle size of copper compounds, according to Torgeson (1967). Compared to using 1, 2, and 3 fungicidal sprays with a 10-day gap between each spray, the study's results showed that using 4 fungicidal sprays is comparatively more successful in controlling anthracnose. According to Crump (2009), a similar impact of copper sprays is recognised to be the most effective at controlling anthracnose and the treatment must be done at the correct time with a shorter duration. The use of fewer sprays at a lower dosage of 0.2% resulted in a higher percentage of disease incidence and intensity of anthracnose, which could be attributed to a quick increase in disease inoculum. Because copper fungicides had little curative effect, the disease spread quickly once the infection was established in the plants. Timmer *et al.* (1996) showed that longer time intervals between spraying result in an inability of the fungicide to redistribute over new foliar growth as well as weathering loss, and therefore protective coverage against the pathogen is lost over time. In this study, plots treated with 4 sprays of Copper Oxychloride at 10-day intervals had the maximum healthy pumpkin output (1.78 t/ha) and the lowest disease intensity (22.20%). The increased fruit yield of this spray schedule over the other spraying schedules can be attributed to more sprays at 10-day intervals, which hampered the establishment of the anthracnose pathogen on the foliage, hence reducing the development of the disease infestation. The current findings are consistent with the findings of Marine *et al.* (2016), who indicated that cucurbit yield loss is based on the amount of green foliage affected by the illness, which is dependent on the onset of the disease and the rate at which necrotic foliage rises. Gent *et al.* (2003) also recorded an increase in yield when the foliar diseases of vegetable crops were treated with bio-formulations in association with copper fungicides.

Table 1: *In-vitro* efficacy of three forms of copper fungicide on mycelia growth and percent inhibition of *C. lagenarium.*

Treatments	Mycelial growth* (mm)				Percent inhibition (%)			
	10ppm	25ppm	50ppm	100ppm	10ppm	25ppm	50ppm	100ppm
Copper oxychloride 50% WP (TRUCOP 50% WP)	51.07 (45.80)^{d}	43.11 (41.03)^{d}	36.67 (37.25)^{d}	25.11 (30.06)^{c}	43.25%	52.10%	59.25%	72.10%
Copper hydroxide 53.8% w/w(KOCIDE 2000)	56.92 (48.98)^{c}	47.47 (43.54)^{c}	44.10 (41.61)^{c}	32.71 (31.74)^{c}	36.75%	47.25%	51.00%	63.65%
Bordeaux mixture	73.35 (58.92)^{b}	63.90 (53.07)^{b}	59.71 (50.60)^{b}	58.27 (49.76)^{b}	18.50%	29.00%	33.65%	35.25%
Control	90.00 (71.56)^{a}	90.00 (71.56)^{a}	90.00 (71.56)^{a}	90.00 (71.56)^{a}	-	-	-	-
SEd (±)	1.032	1.075	0.990	0.874	-	-	-	-
CD (0.05)	2.417	2.485	2.318	2.048	-	-	-	-

Table 2: Comparative efficacy of Copper Oxychloride 50% WP fungicide on disease incidence of anthracnose of pumpkin.

Treatments	Percent incidence of anthracnose of pumpkin at different intervals (days after first appearance of symptoms)					
First appearance of disease 10.12.2021	0 Days	7 days	14 Days	21 Days	28 Days	35 days
T_1: 2 numbers of spray @ 0.2% at 10 days interval	16.70 (24.12)^{a}	33.30 (35.24)^{b}	38.87 (38.49)^{b}	50.00 (45.00)^{b}	66.70 (54.75)^{b}	77.77 (65.88)^{b}
T_2: 3 numbers of spray @ 0.2% at 10 days interval	16.70 (24.12)^{a}	27.77 (31.53)^{cd}	33.30 (35.24)^{bc}	44.43 (41.748)^{bc}	61.13 (51.50)^{bc}	72.23 (58.46)^{c}
T_3: 4 numbers of spray @ 0.2% at 10 days interval	11.13 (16.47)^{a}	22.23 (27.83)^{cd}	27.77 (31.53)^{cd}	38.87 (38.496)^{bc}	55.57 (48.25)^{c}	66.70 (54.75)^{cd}
T_4: 2 numbers of spray @ 0.25% at 10 days interval	16.70 (24.12)^{a}	16.70 (24.12)^{d}	22.23 (27.82)^{de}	33.30 (31.536)^{cd}	44.43 (41.74.)^{d}	61.13 (48.25)^{de}
T_5: 3 numbers of spray @ 0.25% at 10 days interval	11.13 (16.47)^{a}	16.70 (24.12)^{d}	27.77 (27.82)^{de}	27.77 (31.536)^{cd}	38.87 (38.49)^{de}	50.00 (45.00)^{ef}
T_6: 4 numbers of spray @ 0.25% at 10 days interval	11.13 (16.47)^{a}	16.70 (24.12)^{d}	16.70 (24.12)^{e}	22.23 (27.828)^{d}	33.30 (35.24)^{e}	38.87 (38.49)^{f}
T_7: Absolute Control (Unsprayed)	16.70 (24.12)^{a}	50.00 (45.00)^{a}	72.23 (58.464)^{a}	88.87 (54.760)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}
SED (±)	N/S	2.682	2.648	4.450	2.837	3.048
CD (0.05)	N/S	5.848	6.595	8.287	6.185	6.856

Values in the parentheses are angular transformed values; Values superscripted with same letter are not significantly different (P=0.05)

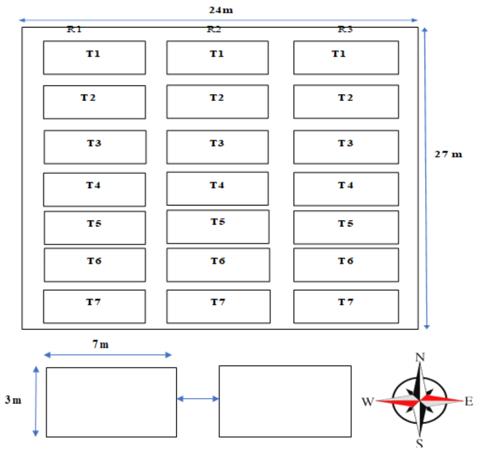

Fig. 1. Layout of experimental plot for anthracnose of pumpkin.

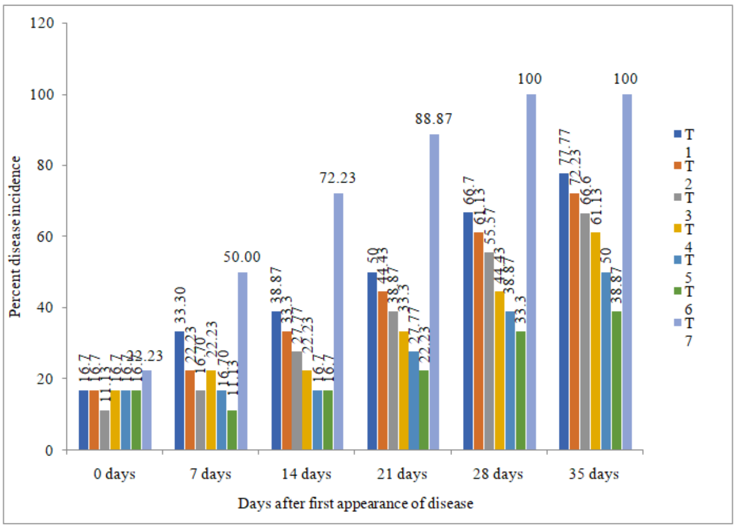

Fig. 2. Comparative efficacy of Copper Oxychloride 50% WP fungicide on disease incidence of anthracnose of pumpkin.

CONCLUSIONS

In conclusion, the anthracnose of pumpkin is one of the most important diseases of pumpkin caused by *Colletotrichum lagenarium*. Since, the National Center of Organic Farming, Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India permits the use of copper fungicides in organic agriculture with a restriction of 8 kg/ha/year, copper fungicides can be used as an important tool for effective management of the disease in pumpkin cultivation under the organic situation. Hence, the use of copper fungicides at different concentrations and with an effective spray schedule has been attempted in the current study. The result of the present study concluded that 4 number sprays of Copper Oxychloride @0.25% at 10 day intervals is highly effective for the management of anthracnose in pumpkin under organic cultivation.

FUTURE SCOPE

The findings of this study can be used to develop different numbers of spray schedules of copper-based fungicides, bio-pesticides and bio-fertilizers that may be tested against the disease for effective management.

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Conflict of Interest. None.

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