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Evaluation of the efficiency of three fungicides on radial mycelial growth, sporulation and germination of *Curvularia lunata*, a rice curvulariosis disease in Burkina Faso

Abalo Itolou KASSANKOGNO[1*], Abdoul Kader GUIGMA[2], Kadidia KOITA[2], Abdoulaye NANA[3] and Issa WONNI[1]

[1]Laboratoire de Phytopathologie, Centre National de la Recherche Scientifique et Technologique/Institut de l'Environnement et de Recherches Agricoles/ BP910 Bobo Dioulasso, Burkina Faso.
[2]Biosciences Laboratory, Department of Plant Biology and Physiology, Joseph KI-ZERBO University, UFR/SVT, 03 BP7021 Ouagadougou, Burkina Faso
[3]Ministry of Agriculture, National Agricultural Training School of Matourkou (ENAFA-Matourkou), BP130 Bobo-Dioulasso, Burkina Faso.

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Rice curvulariosis caused by *Curvularia lunata* is an emerging disease in Burkina Faso. This study evaluated the efficiency of three contact fungicides on the developmental stages of this pathogen *in vitro*. The experimental design used was a split-plot. The main factor included fungicides with three modalities, and the secondary factor was the doses of fungicides with four levels, including the untreated control, which was repeated four times. The fungal material consisted of the *Curvularia lunata* (BM) strain. The fungicides used were Copper Hydroxide, Mancozeb and Chlorothalonil. The results showed a very good efficiency of Mancozeb (PI=100%) and Copper Hydroxide (PI>90%) on mycelial growth, sporulation and germination of conidia of *Curvularia lunata* while Chlorothalonil obtained a medium efficiency on mycelial growth (PI=64.2%), a very low efficiency on sporulation (PI=33.25%) and a good efficiency on conidial germination (PI=79.31%). These results are relevant and require a complementary study to evaluate the effectiveness of these fungicides on the manifestations of curvulariosis on the plant in order to design an effective control method.

Key words: Fungicides, curvulariosis, *Curvularia lunata*, rice, *in vitro*, efficency.

INTRODUCTION

Rice is the second cereal produced in the world and is the staple food for more than half of the world's population (FAOSTAT, 2014). According to Mendez Del Villar (2022), world production in 2021 was 782 million

*Corresponding author. Email: kassabalo@yahoo.fr.

Table 1. Characteristics of the fungicides tested.

No.	Trade name	Composition	Doses	Mode of operation
1	IDEFIX	Copper hydroxide, 65.5%, WP	3 kg/ha	Contact
2	MANGA-PLUS	Mancozeb, 800 g/Kg, WP	2 kg/ha	Contact
3	TALO	Chlorothalonil, 720 g/L, SC	1.5 l/ha	Contact

Source: PROPHYMA/SAVANA SA (2022).

tons compared to 776.7 million tons in 2020. In sub-Saharan Africa, rice represents 25% of cereals consumed, with an average per capita consumption of 45kg /person/year (Mendez Del Villar, 2017). In Burkina Faso, rice has become a staple crop and ranks 4th among cereals in terms of cultivated area and production. It is produced throughout the national territory and represents 8% of total cereal production, with 5% of total cultivated areas (Koutou et al., 2021). National production for the 2021-2022 crop years was estimated at 451.421 tons (MAAH, 2022). However, this production covers less than 50% of the needs of the populations (MICA, 2020). Indeed, the crop is faced with multiple abiotic and biotic constraints that lead to significant drop in production (Ouedraogo and Dakouo, 2017). Among the biotic constraints, fungi such as *Pyricularia oryzae*, *Bipolaris oryzae* and *Curvularia lunata* remain the most harmful and compromise the rice harvest in quantity and/or quality (Wopereis et al., 2008; Imrani et al., 2014). *Curvularia lunata* (Walkker) Boedijn, which causes rice curvulariosis, is one of the fungal pathogens frequently found on rice seeds (Ouedraogo et al., 2016). This disease is increasing in extent, leading to leaf and stem damage and seed deterioration (Imrani et al., 2014). However, few studies have been conducted to better understand the populations of this emerging pathogen and to identify effective means of control. The management of cryptogamic and bacteriological diseases of fruit, vegetable and cereal crops, in Burkina Faso, producers uses broad-spectrum fungicides such as Mancozeb, Copper hydroxide and Chlorothalonil. However, very few studies have been conducted on the effectiveness of these fungicides on *Curvularia lunata*. It is therefore important to conduct this study, the overall objective of which is to contribute to the establishment of an effective control method against rice curvulariosis in Burkina Faso. Specifically, it will compare the effectiveness of Copper Hydroxide, Mancozeb and Chlorothalonil on radial mycelial growth, sporulation and *Curvularia lunata* germination in the laboratory.

MATERIALS AND METHODS

Site description

The work was carried out under controlled conditions within the research station of the Institute for the Environment and Agricultural Research (INERA) in Farako-Bâ, located 10 km south-west of Bobo-Dioulasso on the Bobo-Banfora. Its geographical coordinates are 04°20' west longitude, 11°60' north latitude, with an altitude of 405 m.

Fungal material and fungicides

The fungal material used consisted of the strain of *Curvularia lunata* called BM which was isolated in 2019 and stored in the plant pathology laboratory of the rice and rice growing program at the Farako-Bâ research station. Three fungicides from PROPHYMA/SAVANA SA were used in this study, the characteristics of which are given in Table 1.

Experimental design

The experimental design used was a split-plot with 2 factors: the main factor being the fungicides with 3 modalities and the dose representing the secondary factor with 3 levels plus the untreated control and repeated 4 times.

The three levels of fungicides are defined according to the standards of the Sahelian Pesticides Committee, considering 3/4 and 5/4 of the dose recommended against fungal diseases of vegetable crops by the production company respectively as the lower dose and the dose superior (COAHP, 2014). The characteristics of the different treatments are shown in Table 2.

Preparation of poisoned culture media

The culture medium was prepared using 42 g of PDA per 1000 mL of distilled water. This mixture was homogenized on a hot plate before being sterilized in an autoclave at 120°C for 30 minutes. After cooling to a temperature of around 50°C (Kassankogno et al., 2021), the different doses of fungicides were incorporated into the culture medium and homogenized. The untreated control consisted of PDA medium without fungicide. This mixture obtained was poured under the extractor hood, into petri dishes at the rate of 25 ml per petri dish. For each dose, including the untreated control, 4 petri dishes were used.

Assessment of radial growth

A 5 mm diameter mycelial explant, 8 days old was removed using a cookie cutter and placed in the center of the Petri dishes containing the different treatments. These petri dishes were placed in an incubator at a temperature of 28°C and in the dark, favorable conditions for the development of the fungus (Hassikou et al., 2002). Mycelial growth was evaluated by measuring the average diameter of the colonies on the 2nd, 4th and 6th Day After Seeding (DAS). The percentage inhibition of mycelial growth compared to controls was calculated using the data from the last evaluation according to the formula of Serghat et al. (2004):

Table 2. Characteristics of the different treatments.

Trade name	Active ingredients/formulation	Treatments	Doses
Control	-	Sterile distilled water	-
IDEFIX	Copper hydroxide, 65.5%, WP	Lower dose	7.5 g/L
		Recommended dose	10 g/L
		Higher dose	12.5 g/L
MANGA-PLUS	Mancozeb, 800 g/Kg, WP	Lower dose	5.1 g/L
		Recommended dose	6.7 g/L
		Higher dose	8.4 g/L
TALO	Chlorothalonil, 720 g/L, SC	Lower dose	5.1 ml/L
		Recommended dose	6.7 ml/L
		Higher dose	8.4 ml/L

Source: Author

Table 3. Level of fungicidal efficiency according to the Kumar et al. (2007).

Scale	Class of mycelial inhibition rates I (%)	Level of sensitivity/resistance of the fungus	Fungicide efficiency level
1	I > 90	Highly sensitive	Very good efficiency
2	75% < I ≤ 90	Sensitive	Good efficiency
3	60% < I ≤ 75	Moderately resistant	Average efficiency
4	40% ≤ I ≤ 60	Resistant	Low efficiency
5	I < 40	Highly resistant	Very low efficiency

Source: Kumar et al. (2007)

PIc (%) = $((C_0-C_c)/C_0) \times 100$;

With: C_0: radial growth in the culture medium without fungicide (in cm) C_c: radial growth in the culture medium with the fungicides (in cm).

Evaluation of sporulation on the different treatments

The evaluation of sporulation on the different treatments was done on the 8th using the same dishes used for the measurement of radial growth. A quantity of 10 ml of distilled water is poured into each Petri dish, then scraped lightly to release the conidia. The suspension was placed in a cryotube and vortexed for 30 second at 2500 rpm to detach the conidia from the conidiophores. The resulting solution was filtered to separate the mycelial fragments from the conidia. These conidia were counted using a Malassez cell. For each suspension, 5 counts in 10 squares of the cell were made. The conidial concentration was done according to the formula:

C= (Number of spores in 10 squares × 1000) /10

The percentage of inhibition of conidia production (PI) was calculated according to the formula of Serghat et al. (2004) :
PI (%) $=(N_0-N_c) /N_0 \times 100$; with N_o: number of conidia estimated in the control, N_c: number of conidia estimated in the presence of fungicide

Evaluation of conidia germination on the different treatments

For the evaluation of conidial germination, a conidial suspension was collected from a fungal culture and adjusted to 10^5 conidia / ml with distilled water. Then, 0.3 ml of the conidial suspension was spread on the Petri dishes containing the different treatments. These dishes were incubated at 28°C in the dark for 12 to 24 hours. Counting was done on a total of 200 conidia distributed in 4 Petri dishes at the rate of 50 conidia per dish. Counting is done using a “Euromex” microscope fitted with a camera at 12 and 24 hours after inoculation. The percentage of conidial germination inhibition relative to the control was calculated using the data from the last evaluation according to the formula of Serghat et al. (2004).

PI (%) $=(N_0-N_c) /N_0 \times 100$

where: N_o: number of conidia germinated in the culture medium without fungicide, N_c: number of conidia germinated in the medium with fungicides added.

Determination of fungicide efficiency level

The evaluation of the level of fungicide efficiency was done according to the Kumar et al. method (2007). The level of sensitivity or resistance of the strain to fungicides was determined according to the Kumar et al. (2007) scale modified by Yao et al. (2018) (Table 3). The determination of fungicide efficiency level was based on the overall inhibition percentage. For the efficiency of the doses, it was determined based on the percentage of inhibition of each dose.

Data processing and analysis

Data were entered and processed using Excel spreadsheet version

Table 4. Efficiency of fungicides on mycelial radial growth.

Fungicides	RG -Lower dose					RG- Recommended dose					RG-Higher dose					GPI (%)	LFE
	2DAS	4DAS	6DAS	PI(%)	LDE	2DAS	4DAS	6DAS	PI (%)	LDE	2DAS	4DAS	6DAS	PI (%)	LDE		
Control	3.40^{c}	5.93^{c}	9.00^{d}	0	-	3.40^{c}	5.93^{c}	9.00^{d}	0	-	3.40^{c}	5.93^{c}	9.00^{d}	-	-	-	-
Chlorothalonil	1.18^{b}	2.45^{b}	3.30^{c}	63.3	AE	1.42^{b}	2.50^{b}	3.26^{c}	63.7	AE	1.37^{b}	2.63^{b}	3.10^{c}	65.6	AE	64.2	AE
Copper Hydroxide	0.00^{a}	0.45^{a}	0.95^{b}	89.4	GE	0.00^{a}	0.52^{a}	0.53^{b}	94.1	VGE	0.00^{a}	0.27^{a}	0.50^{b}	94.4	VGE	92.7	VGE
Mancozeb	0.00^{a}	0.00^{a}	0.00^{a}	100	VGE	0.00^{a}	0.00^{a}	0.00^{a}	100	VGE	0.00^{a}	0.00^{a}	0.00^{a}	100	VGE	100	VGE
Pr > F	< 0.0001	< 0.0001	< 0.0001	-	-	< 0.0001	< 0.0001	< 0.0001	-	-	< 0.0001	< 0.0001	< 0.0001	-	-	-	-
Significant	VHS	VHS	VHS			VHS	VHS	VHS	-		VHS	VHS	VHS	-		-	-

RG: Radial growth; DAS: Day after seeding; PI: Percentage of inhibition; VHS: Very highly significant; GPI: Global percentage inhibition; LFE: Level of fungicide efficiency; AE: Average efficiency; GE : Good efficiency, VGE: Very good efficiency. Means followed by the same letters in the same column are not significantly different according to the Newman-Keuls statistical test (SNK) at the 5% threshold.
Source: Author

2016. XLSTAT 2016.02.27444 - ANOVA software was used for analysis of variances followed by comparison of means by the Newman-Keuls (SNK) statistical test at the 5% threshold.

RESULTS

Comparison of fungicide efficiency on mycelial radial growth of *Curvularia lunata*

Comparison of the means of mycelial radial growth of *Curvularia lunata* on the 2nd, 4th, and 6th Days after seeding (DAS) showed a very highly significant difference (Pr< 0.0001) between doses and between fungicides (Table 4).

Fungicides exerted inhibition on mycelial radial growth at all evaluation dates. Inhibition increased progressively for all fungicides at all doses except Mancozeb, which was completely inhibitory at the lowest dose. Mycelial radial growth at 6 DAS was higher on the untreated control (9 cm) compared to the Chlorothalonil and Copper Hydroxide treatments which recorded maximum mycelial radial growths of 3.3 cm and 0.95 cm respectively. No growth was observed with the Mancozeb treatments. The percentage of fungicide inhibition varied among fungicides and doses. It was 100% for Mancozeb at all rates at 6 days of age and decreased with rate level for Chlorothalonil and Copper Hydroxide. It was 65.6, 63.7, and 63.3% for Chlorothalonil and 94.4, 94.1, and 89.4% for Copper Hydroxide at the higher, recommended, and lower doses, respectively.

The analysis of the level of fungicide efficiency according to the doses showed that Chlorothalonil was moderately effective for all doses.

Copper Hydroxide had good efficiency at the lower rate and very good efficiency from the recommended rate. Mancozeb showed very good efficiency at all rates. The overall inhibition percentage of the fungicides was 100% for Mancozeb, 99.7% for Copper Hydroxide and 64.2% for Chlorothalonil.

The overall grouping of fungicides according to the level of efficiency resulted in two groups. The group of fungicides of medium efficiency (Chlorothalonil) and the group of fungicides of very good efficiency (Copper Hydroxide and Mancozeb).

Comparison of fungicide efficiency on sporulation of *Curvularia lunata*

Comparison of the means of the number of conidia of *Curvularia lunata* at the 8th DAS showed a very highly significant difference (Pr< 0.0001) between doses and between fungicides (Table 5). Sporulation varied between fungicides and doses. The untreated control had the highest sporulation ($11.8x10^5$ conidia/mL) compared to the others. Sporulation decreased progressively with the Chlorothalonil-based doses with $10.3x10^5$ conidia/mL, $7.9x10^5$ conidia/mL and $4.2x10^5$ conidia/mL corresponding to the lower, recommended and higher doses respectively. No sporulation was observed for the Mancozeb and Copper Hydroxide treatments. The percentage of inhibition was variable between fungicides and doses. The strong inhibitions were observed in the treatments based on Mancozeb (100%) and Copper Hydroxide (100%). As for the treatments

Table 5. Comparison of conidia counts by treatment.

Fongicides	Lower dose			Recommended dose			Higher dose			GPI	LFE
	Conidia	PI (%)	LED	Conidia	PI (%)	LED	Conidia	PI (%)	LED		
Control	11.8×10^{6c}	0	-	11.8×10^{6c}	0	-	11.8×10^{6c}	0	-	-	-
Chlorothalonil	10.3×10^{6b}	12.7	VLE	7.9×10^{6b}	33.3	VLE	0.42×10^{6b}	53.7	LE	33.3	VLE
Copper Hydroxide	0.00^{a}	100	VGE	0.00^{a}	100	VGE	0.00^{a}	100	VGE	100	VGE
Mancozeb	0.00^{a}	100	VGE	0.00^{a}	100	VGE	0.00^{a}	100	VGE	100	VGE
Pr > F	< 0.0001	-	-	< 0.0001	-	-	< 0.0001	-	-	-	-
Significant	VHS	-	-	VHS	-	-	VHS	-	-	-	-

VHS: Very highly significant, VLE: Very low efficiency, LE: low efficiency, LED: Level of effectiveness of the dose, VGE: Very good efficiency. Means followed by the same letters in the same column are not significantly different according to the Newman-Keuls statistical test (SNK) at the 5% threshold.
Source: Author

based on Chlorothalonil, the inhibition was progressive with respectively percentages of inhibition of 12.7, 33.3 and 64.4%, respectively, corresponding to the low dose, the recommended dose, and the superior dose. The analysis of the level of efficiency of the fungicides according to the doses showed that Chlorothalonil has a very low efficiency at the lower and recommended dose and a low efficiency at the higher dose. Copper Hydroxide and Mancozeb had very good efficiency at all rates. The overall ranking of fungicides by efficiency level resulted in the very low efficiency group (Chlorothalonil) and the very good efficiency group (Copper Hydroxide and Mancozeb.

Comparison of fungicide efficiency on conidial germination

Comparison of the means of the number of germinated conidia of *Curvularia lunata* showed a very highly significant difference (Pr< 0.0001) between rates and between fungicides (Table 6). It was higher for the untreated control compared to the others.

Conidial germination was variable between fungicides, doses, and observation time. The untreated control had the highest number of germinated conidia (196/200 conidia). Chlorothalonil treatments recorded at least 46/200 germinated conidia compared to Mancozeb and Copper Hydroxide treatments which completely inhibited germination. Conidial germination on Chlorothalonil treatments was dose dependent with 46/200 conidia, 41/200 conidia and 36/200 conidia corresponding to the number of conidia germinated on the lower, recommended and higher dose respectively. The percentage of inhibition was 100% for Mancozeb and Copper Hydroxide treatments and 75.8% for the lower dose of Chlorothalonil, 79.1% for the recommended dose and 81.6% for the higher dose. The analysis of the level of efficiency of the fungicides according to the doses showed that Chlorothalonil has a good efficiency at all the doses. Mancozeb and Copper Hydroxide had very good efficiency at all rates.The overall ranking of the fungicides according to the level of efficiency resulted in the group of fungicides with good efficiency (Chlorothalonil) and the group of fungicides with very good efficiency (Mancozeb and Copper Hydroxide).

DISCUSSION

The results of this study showed that the fungicides tested inhibited mycelial radial growth, sporulation, and germination of conidia of Curvularia lunata. Inhibition of fungal growth varied depending on the fungicide, dose, and date of evaluation. Indeed, Mancozeb completely inhibited mycelial radial growth at the 6th day of the week, while Chlorothalonil and Copper Hydroxide treatments recorded maximum mycelial radial growths of 3.3 cm and 0.95 cm, respectively. Also, the calculation of inhibition percentages showed that Mancozeb inhibited 100% of mycelial radial growth compared to Chlorothalonil and Copper Hydroxide which partially inhibited radial growth with inhibition Hydroxide. The Mancozeb and Copper Hydroxide percentages ranging from 63 to 65% for Chlorothalonil and 89 to 94% for Copper treatments completely inhibited conidial

Table 6. Comparison of the number of germinated conidia according to the treatments.

Fongicides	Lower dose				Recommended dose				Higher dose				GPI	LFE
	GC 12 HAS	GC 24 HAS	PI (%)	LED	GC 12 HAS	GC 24 HAS	PI (%)	LED	GC 12 HAS	GC 24 HAS	PI (%)	LED		
Control	185^{b}	196^{c}	0	-	185^{b}	196^{c}	0	-	185^{b}	196^{c}	0	-	-	-
Chlorothalonil	0^{a}	46^{b}	76.5	GE	0^{a}	41^{b}	79.8	GE	0^{a}	36^{b}	81.6	GE	79.3	GE
Copper Hydroxide	0^{a}	0^{a}	100	VGE	0^{a}	0^{a}	100	VGE	0^{a}	0^{a}	100	VGE	100	VGE
Mancozeb	0^{a}	0^{a}	100	VGE	0^{a}	0^{a}	100	VGE	0^{a}	0^{a}	100	VGE	100	VGE
Pr > F	< 0.0001	< 0.0001	-	-	< 0.0001	< 0.0001	-	-	< 0.0001	< 0.0001	-	-	-	-
Significant	VHS	VHS	-	-	VHS	VHS	-	-	VHS	VHS	-	-	-	-

LED: Level of effectiveness of the dose, GPI: Global percentage inhibition; GC: Germinated conidia; HAS: Hour after seeding; PI: Percentage of Inhibition; VHS: Very Highly significant , LFE: Level of fungicide efficiency; GE: Good efficiency; VGE: Very good efficiency. Means followed by the same letters in the same column are not significantly different according to the Newman-Keuls statistical test (SNK) at the 5% threshold.
Source: Author

production and germination while the Chlorothalonil treatments were dose-dependent. In fact, the control recorded a conidial production of 11.8×10^5 conidia/mL against a conidial production of 10.3×10^5 conidia/mL, 7.9×10^5 conidia/mL and 4.2×10^5 conidia/mL corresponding respectively at the lower dose, the recommended dose and the higher dose of Chlorothalonil. The inhibition of conidia was due to the fact that copper was toxic to fungal spores and to be effective it must be applied preventively.

The percentage inhibition of conidial germination was 100% for Mancozeb and Copper Hydroxide treatments and 75.8% for the lower dose of Chlorothalonil, 79.1% for its recommended dose and 81.6 for its higher dose. This strong inhibition of Mancozeb and Copper Hydroxide on mycelial radial growth, sporulation and germination of conidia of *Curvularia lunata* would not only be a function of the active ingredient but also of its concentration in the culture medium. Indeed, according to Leroux (2003), Mancozeb and Copper Hydroxide are "multi-site" fungicides which act mainly on respiratory processes in the pathogen due to the blocking of enzymes or coenzymes with thiol groups. These results are comparable to those of Tenon et al. (2017) who showed that Mancozeb 100% inhibits mycelial growth, sporulation and germination of *Colletotrichum gloeosporioides* causative agent of cashew anthracnose in Benin. A similar study performed *in vitro* by Karmakar et al. (2016) on the efficiency of 7 fungicides including Sprint (Carbendazim 25% + Mancozeb 50%WS) and Merger (Tricyclazole 18% +Mancozeb 62% WP) showed that these active ingredients resulted in complete inhibition of mycelial growth of *Helminthosporium oryzae, Alternaria padwickii, Curvularia lunata, Fusarium moniliforme* and *Sarocladium oryza*, all pathogens responsible for discoloration of rice grains. Also, a study by Hajano et al. (2012) on 5 fungicides (Thiophanate-methyl, Carbendazim, Fosesetyl-aluminum, Mancozeb, and copper oxychloride) showed that Mancozeb completely inhibited the mycelial growth of *Magnaporthe oryzae*, a fungus responsible for rice blast. The maximum inhibition of Chlorothalonil was 65.6% on mycelial radial growth. These results are similar to those performed by Yao et al. (2018) who showed that this active ingredient had average efficiency on Corynespora cassiicola, the agent responsible for leaf fall in rubber trees, with average inhibition percentages of 73.3%. Through these results, the lower dose of Mancozeb which completely inhibited mycelial growth, sporulation and germination of Curvularia conidia was the most effective dose.

Conclusion

The study enabled the researchers to determine the efficacy levels of the fungicides and the effective doses of each one.The fungicides tested had inhibitory effects on mycelial growth, sporulation and germination of conidia of *Curvularia lunata.* Chlorothalonil treatments had low efficiency on mycelial growth, sporulation and good efficiency on conidial germination.

Mancozeb and copper hydroxide had very good efficiency on mycelial growth, sporulation and conidial germination. Mancozeb was more effective and the most effective rate was its lower rate (5.1g/L). However, in order to protect the

environment, it would be good to make a range of Mancozebe fungicide concentrations to determine a minimum effective dose. Similarly, a complementary field study on the efficacy of these two fungicides in preventive and curative treatment is necessary to confirm the efficacy of these fungicides on rice curvulariosis in the field.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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