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Management of purple blotch disease on onion (*Allium cepa* L.) using fungicides and plant-based pesticides in West Africa, Burkina Faso

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ABSTRACT: Onion *(Allium cepa* L.) purple blotch caused by *Alternaria porri* (Ellis) Cif. is a disease of world-wide importance. The aim of this study is to evaluate the effectiveness of treatment combinations with fungicides and plant-based pesticides in the control of *A. porri,* onion growth and bulb production. To do so, the effect of aqueous extracts (5%, 10%, 15%) of *Lippia multiflora, Azadirachta indica* and the fungicides Mancozeb 80 WP (1g/L) and Azoxystrobin 250 SC (0.2%) on the radial growth of *A. porri* on Potato Dextrose Agar (PDA) medium was assessed. Furthermore, the onion variety «Prema» was used in field in a Fisher block design with four replicates. The treatments included the following: (i) the application of foliar sprays with distilled water (control), Mancozeb at 2 kg/ha and Azoxystrobin (1L/ha); (ii) the ground application of plant leaf powder (400 g/m^2) at seven days before transplanting, which was then followed by the application of aqueous plant extracts and fungicides as foliar sprays. Sprays were carried out at 60, 80 and 100 days after transplanting. The results show that treatments inhibited the growth of *A. porri* from 25.35 to 100%. Furthermore, bulb yields in the plots treated with plant powders, ranging from 35 to 43.25 t/ha, exceeded that of the fungicide Mancozeb, which was 28.75 t/ha. From these findings, soil and leaf treatments with plant extracts may be used in the control of onion purple blotch disease. Nevertheless, it is essential to study the effects of plant extracts on soil fauna and microorganisms.

KEYWORDS: Onion, purple blotch, fungicide, plant extract, *Alternaria porri*.

1 INTRODUCTION

Onions (*Allium cepa* L.) are the most important vegetable crop in Burkina Faso, both in terms of area and yield [1]. Six regions account for 80% of Burkina Faso's bulb onion production [2]. These regions are the North (65,384 tonnes), Hauts-Bassins (29,968 tons), Boucle du Mouhoun (27,049 tons), Centre-West (26,725 tons), Central Plateau (22,538 tons), and North Center (21,696 tons). According to [3], the onion sector in Burkina Faso provides employment for about 400,000 people, including 100,000 women. The onion is a basic ingredient in a large number of culinary dishes [4]. It is appreciated for its unique aroma [4]. The medicinal properties of onions are numerous, and they have been used in medicine since ancient times [5]. Onion extract is known for its antibacterial, antifungal, anti-helminthic, anti-inflammatory, anti-septic, and anti-spasmodic properties [6]. According to [7], the colourful bulbs are a natural source of antioxidants.

Onion cultivation in Burkina Faso is affected by various abiotic factors, including inadequate water management and soil conditions that can adversely affect crop yields and bulb quality. In addition, onion production in Burkina Faso is impacted by various phytosanitary constraints. Inventory studies have identified the primary fungal diseases affecting onion crops as purple blotch disease, caused by *Alternaria porri,* and onion basal rot or *Fusarium* head blight, caused by *Fusarium oxysporum* or *Fusarium solani* [8]. In Burkina Faso, onion purple blotch disease is the most prevalent and widespread fungal disease [8]. Purple blotch disease results in significant yield losses in the field and leads to bulb rot during storage, according to research by [9]. For example, this disease was reported to cause over 60% yield losses from India [10], [11]. In addition to reducing

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yields, *Alternaria porri* also produces mycotoxins in onion leaves that pose a health hazard for both humans and farm animals [8]. According to [12], the risk is particularly high in Burkina Faso, where onion leaves are consumed raw or processed by local populations.

To control this disease, several fungicides several fungicides found to be effective worldwide include chlorothalonil 75% WP, Mancozeb 75% WP, propineb 70% WP, difenconazole 25% EC, propiconazole 25% EC, hexaconazole 5% EC, Rovral 50 WP (0.2%), Dithane M-45 80 WP (0.2%) [13]. Biopesticides, including aqueous extracts of *Azadirachta indica, Bougainvillea sp.,* and *Cymbopogon citratus* have demonstrated effectiveness against *A. porri* in studies conducted by [14] and [15]. These fungicides and biopesticides are used as foliar sprays to treat onion purple spot. However, it is important to note that the primary inoculum of the disease may persist in the soil and crop debris [16], [17]. Thus, soil treatments could potentially reduce the primary inoculum of *A. porri*. The present study aims to assess the effectiveness of treatment combinations with plant-based and chemical fungicides in controlling *A. porri* and their impact on onion growth and yield.

2 MATERIALS AND METHODS

2.1 ASSESSING THE EFFECT OF AQUEOUS PLANT EXTRACTS AND FUNGICIDES ON THE RADIAL GROWTH OF A. PORRI IN VITRO

To assess the effect of *L. multiflora* and *A. indica* extracts on the radial growth of *A. porri,* three different concentrations of aqueous extract were prepared with the addition of 5g, 10g and 15g of leaf powder to 100ml of distilled water, respectively. The mixture was stirred at 250 rpm for 24 hours with a magnetic stirrer and then filtered through a fine mesh (5 µm) to achieve 5%, 10%, and 15% concentrations of aqueous extract. Then 3,15 Potato Dextrose Agar (PDA) was dissolved in 75 ml of the extract at the concentrations indicated above. The resultant solution underwent sterilization in an autoclave at a temperature of 121°C for 15 minutes. After cooling, 0.25 g of streptomycin was added to every solution, which was homogenized and then distributed into Petri dishes at a rate of 15 ml per dish. Fungicides were added to the PDA medium at 1g/L for Mancozeb and 0.2% for Azoxystrobin. In the control, *A. porri* was cultured on PDA medium alone. The experiment was repeated four times. The *A. porri* isolate used in this experiment was provided by the Phytopathology and Tropical Mycology Team at the Joseph KI-ZERBO University in Ouagadougou, Burkina Faso. In practice, a four-millimeter explant, taken from a seven-day-old *A. porri* isolate culture on PDA medium using a cookie cutter, is placed aseptically at the center of a Petri dish with the above-prepared culture media. The Petri dishes were sealed with parafilm paper and incubated for seven days at a temperature between 25 and 30°C under a 12: 12 light/dark photoperiod. Two perpendicular straight lines were drawn on the back of the Petri dish passing through the center, and the growth diameters were measured using a slat on each straight line at the 7th day after incubation (DAI) [8]. The mean growth diameter was the result of the measurement of two perpendicular lines. Finally, we calculated the rate of inhibition of mycelial growth by phytosanitary products using formula 1 from [18].

$$RI = \frac{Dc-Dt}{Dc} \times 100 \qquad \text{(Formula 1)}$$

Where RI = rate of inhibition, Dc = Colony diameter in the water control, Dt = Colony diameter in a treatment.

2.2 EVALUATION OF THE EFFICACY OF TREATMENTS FOR THE CONTROL OF PURPLE SPOT ON ONIONS IN THE FIELD

2.2.1 DESCRIPTION OF THE STUDY SITE AND EXPERIMENTAL DESIGN

In order to assess the effectiveness of fungicides and botanical pesticides in controlling the onion purple blotch disease, we carried out a trial comprising nine distinct treatments (Table 1). Treatments were replicated four times in a Fisher block design. The treatments were replicated four times using a Fisher Block Design, resulting in a total of 36 plots. Each individual plot measured 2m × 0.5 m. The spacing between adjacent repetitions was 1.5 m, with 1 m between elementary plots. Mounding was done around each plot to retain the products applied to the plot. The trial was carried out in the market garden area of a locality named Tabtenga, after careful preparation of the soil. Tabtenga is a village in the Loumbila commune, located in the Oubritenga province of Burkina Faso (Figure 1). The site is situated 25 kilometres away from Ouagadougou, the capital city. The climate in this area is Sudano-Sahelian with an annual rainfall ranging between 600mm and 900mm. Moreover, the soil type in Tabtenga is ferralitic, which is moderately fertile and rich in organic matter.

Table 1. Description of the treatments applied

Codes	Treatments applied
T0 (Control)	Spraying plants with water
T1	Applying *A. indica* powder to the soil, followed by the application of *A. indica* aqueous extract to the plants.
T2	Applying *A. indica* powder to the soil, followed by application of Azoxystrobin fungicide to the plants.
T3	Applying *A. indica* powder to the soil, followed by application of Mancozeb fungicide to the plants.
T4	Applying *L. multiflora* powder to the soil, followed by the application of *L. multiflora* aqueous extract to the plants.
T5	Applying *L. multiflora* powder to the soil, followed by application of Azoxystrobin fungicide to the plants.
T6	Applying *L.multiflora* powder to the soil, followed by application of Mancozeb fungicide to the plants.
T7	Spraying plants with Azoxystrobin fungicide
T8	Spraying plants with Mancozeb fungicide

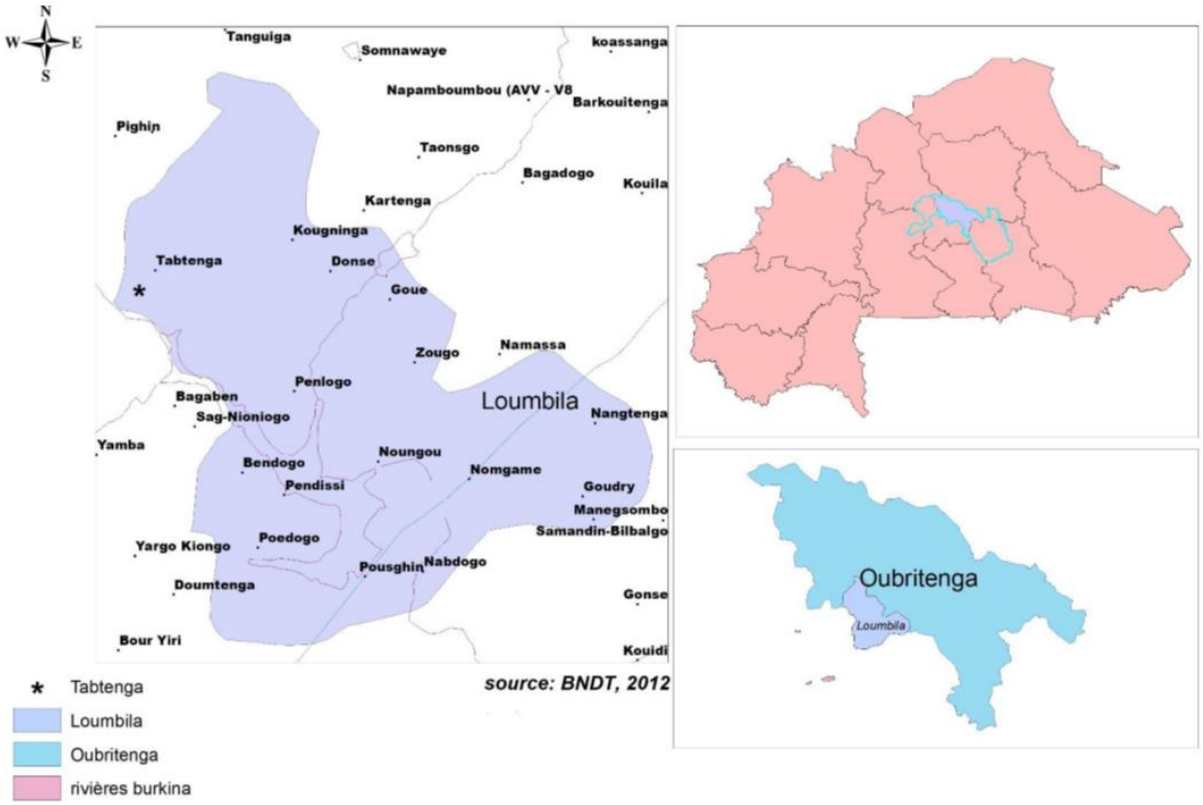

Fig. 1. Location Map of Tabtenga site

2.2.2 Plant Powder Preparation And Soil Application

In order to obtain leaf powders of *A. indica* and *L. multiflora,* we collected leaves from both species and dried them in the laboratory at 25°-30°C for four weeks. The dried leaves were then ground to a fine powder in a mill and preserved in the laboratory. Seven days before transplanting the onion seedlings, the leaf powders were manually spread on the relevant plots at a rate of 400g per m^2. The powder was mixed with the soil through ploughing with a daba. Finally, all plots were watered immediately after weeding, then every two days until the date of transplanting the seedlings.

2.2.3 Transplantation Of Seedlings And Crop Maintenance

In each elementary plot previously watered, 45-day-old seedlings from the nursery were manually transplanted into three rows of two meters each. The spacing between rows was 0.25 m and the spacing between seedlings in the same row was 0.10 m. The trial was irrigated every other day through a piping system after transplantation. All plots received NPK 15-15-15 fertilizer (400 kg/ha) and urea (200 kg/ha) at 14 days and 60 days after transplanting, respectively.

2.2.4 PREPARING AND SPRAYING PHYTOSANITARY PRODUCTS

Aqueous extracts of *A. indica* and *L. multiflora* were prepared by macerating the leaf powder in distilled water at 50 g per liter for 30 minutes [19]. The macerate obtained was then filtered through a fine mesh sieve with five micrometers pores. Plants were sprayed with aqueous extracts using a hand sprayer. The treatments were applied in the morning between 6 a.m. and 9 a.m. to avoid heat which could affect the stability of the extract [19]. Sprays were carried out at 60, 80 and 100 days after transplanting. The fungicides were applied at a rate of 2 kg/ha for Mancozeb and 1 L/ha for Azoxystrobin. Control plants were sprayed with water.

2.3 DATA COLLECTION AND STATISTICAL ANALYSIS

Data collected included seedling recovery after transplanting, number and length of leaves, severity of purple blotch and onion yield. Seedling recovery was assessed at 14 DAI by calculating the percentage ratio between the number of recovered seedlings and the total number of seedlings per elementary plot. Plant height and number of leaves were evaluated on a sample of 10 plants per elementary plot at 45 and 80 DAI. Plant height was measured using a ruler. For yield estimation, the weight of the onions harvested from each elementary plot was determined using a mechanical pan balance. The yield was then calculated in tons per hectare. Disease severity was assessed at 100 DAT using the 1-9 rating scale adapted from [20]. To do so, 10 plants were selected in each plot and the disease severity index was calculated using formula 2 according to [5].

All data were submitted to analysis of variance based on split plot design using XLSTAT 2016 software and the difference between means compared using Duncan's Multiple Range Test at 5%.

3 RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 EFFECT OF AQUEOUS PLANT EXTRACTS AND SYNTHETIC FUNGICIDES ON THE GROWTH OF A. PORRI IN VITRO

The mean diameter of *A. porri* colonies varied between treatments (Table 2). Statistical analysis showed that there was a significant difference (p = 0.0005) between treatments. All treatments had lower mean colony diameters than the control. Growth inhibition rates relative to the control ranged from 25.35% to 54.04% in the aqueous extract treatments and from 64.4% to 100% in the synthetic fungicide treatments. Only the Mancozeb fungicide resulted in complete inhibition of pathogen growth. For the two aqueous extracts used, the 15% dose was the most effective. This was followed by the 10% dose and then the 5% dose.

Table 2. Effect of aqueous plant extracts and synthetic fungicides on the growth of A. porri in vitro

Treatments	Mean colony diameter (cm)	Rate of radial growth inhibition (%)
Control	8.97±0.05^{a}	00.0±0.00^{i}
Azoxystrobin 250 SC	3.37±0.28^{h}	62.4±3.06^{b}
Mancozeb 80 WP	0.00±0.00^{i}	100±0.00^{a}
L. multiflora 5%	6.44±0.11^{c}	28.28±1.23^{g}
L. multiflora 10%	5.47±0.17^{e}	39.00±1.90^{e}
L. multiflora 15%	4.12±0.10^{g}	54.04±1.06^{c}
A. indica 5%	6.70±0.08^{b}	25.35±0.91^{h}
A. indica 10%	5.82±0.05^{d}	33.30±0.56^{f}
A. indica 15%	5.07±0.15^{f}	35.10±1.67^{d}
p value	< 0.0001	

Column means (±SD) followed by the same letter are not significantly different at p = 0.05 according to Duncan's Multiple Range Test.

3.1.2 SEEDLING RECOVERY RATE

Figure 2 shows the recovery rate of seedlings at 14 DAT as a function of treatment. Statistical analysis showed no significant difference (p > 0.05) between the recovery rates in the plant powder treated plots and those in the control plots. The mean recovery rate ranged from 98.41 to 100%.

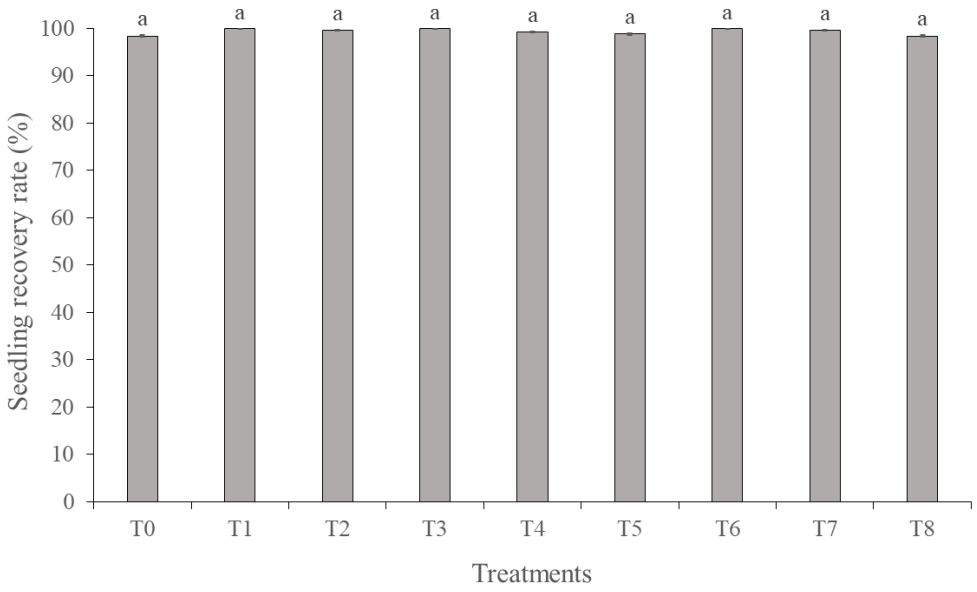

Fig. 2. Seedling recovery rate at 14 DAT depending on the treatments

Bars are means ±SD. Bars with the same letter are not significantly different at p = 0.05 according to Duncan's Multiple Range Test. T0: control, T1: Soil treated with A. indica powder and leaves treated with aqueous extract of A. indica, T2: Soil treated with A. indica powder and leaves treated with Azoxystrobin, T3: soil treated with A. indica powder and leaves treated with Mancozeb, T4: Soil treated with L. multiflora powder and leaves treated with aqueous extract of L. multiflora, T5: soil treated with L. multiflora powder and leaves treated with Azoxystrobin, T6: soil treated with L. multiflora powder and leaves treated with Mancozeb, T7: leaves treated with Azoxystrobin, T8: leaves treated with Mancozeb.

3.1.3 PLANT HEIGHT AND NUMBER OF LEAVES

The average plant height at 45 DAT and 80 DAT ranged from 36.7 to 44.15cm, and 48.70 to 56.12cm, respectively (Table 3). Statistical analysis showed that there was a significant difference ($p \leq 0.0004$) between treatments. The highest plant height was recorded in plots treated with *A. indica* powder and Azoxystrobin (T2) at 45 DAT and 80 DAT. Plant height in plots treated with *A. indica* powder and synthetic fungicides (T2 and T3) was higher than in plots treated with synthetic fungicides alone (T7 and T8).

For the number of leaves per plant, statistical analysis showed no significant difference ($p > 0.05$) between treatments. The average number of leaves per plant ranged from 6.08 to 6.75 at 45 DAT and from 8.55 to 9.62 at 80 DAT.

Table 3. Plant height and number of leaves according to treatments

Treatments	Plant height (cm)		Number of leaves per plant	
	45 DAT	80 DAT	45 DAT	80 DAT
T0	40.92 ± 1.91^{bc}	52.02 ± 2.45^{bc}	6.42 ± 0.35^{a}	9.53 ± 0.29^{a}
T1	38.85 ± 2.39^{cd}	52.25 ± 3.01^{bc}	6.18 ± 0.10^{a}	8.78 ± 0.74^{a}
T2	44.15 ± 2.85^{a}	56.12 ± 2.68^{a}	6.40 ± 0.67^{a}	8.90 ± 0.98^{a}
T3	42.62 ± 0.19^{ab}	54.95 ± 1.10^{ab}	6.75 ± 0.74^{a}	9.62 ± 1.02^{a}
T4	38.2 ± 0.14^{cd}	50.38 ± 1.89^{cd}	6.30 ± 0.45^{a}	8.62 ± 0.46^{a}
T5	36.65 ± 1.48^{d}	54.73 ± 3.70^{ab}	6.10 ± 0.44^{a}	8.55 ± 1.07^{a}
T6	40.75 ± 2.95^{bc}	49.38 ± 2.26^{cd}	6.08 ± 0.54^{a}	8.98 ± 0.59^{a}
T7	36.98 ± 1.54^{d}	$50.60 \pm 1.14b^{cd}$	6.18 ± 0.13^{a}	9.10 ± 0.36^{a}
T8	36.7 ± 2.98^{d}	48.70 ± 2.72^{d}	6.25 ± 0.33^{a}	8.80 ± 0.55^{a}
P. values	0.0004	< 0.0001	0.541	0.349

Bars are means ±SD. Bars with the same letter are not significantly different at p = 0.05 according to Duncan's Multiple Range Test. T0: control, T1: Soil treated with A. indica powder and leaves treated with aqueous extract of A. indica, T2: Soil treated with A. indica powder and leaves treated with Azoxystrobin, T3: soil treated with A. indica powder and leaves treated with Mancozeb, T4: Soil treated with L. multiflora

powder and leaves treated with aqueous extract of L. multiflora, T5: soil treated with L. multiflora powder and leaves treated with Azoxystrobin, T6: soil treated with L. multiflora powder and leaves treated with Mancozeb, T7: leaves treated with Azoxystrobin, T8: leaves treated with Mancozeb.

3.1.4 SEVERITY OF ONION PURPLE BLOTCH DISEASE

Figure 3 illustrates the severity of the purple leaf blotch disease at 100 days after treatment (DAT). A statistical analysis revealed no significant difference (p>0.05) between the treatments, and the disease severity ranged from 13.89% to 16.67%.

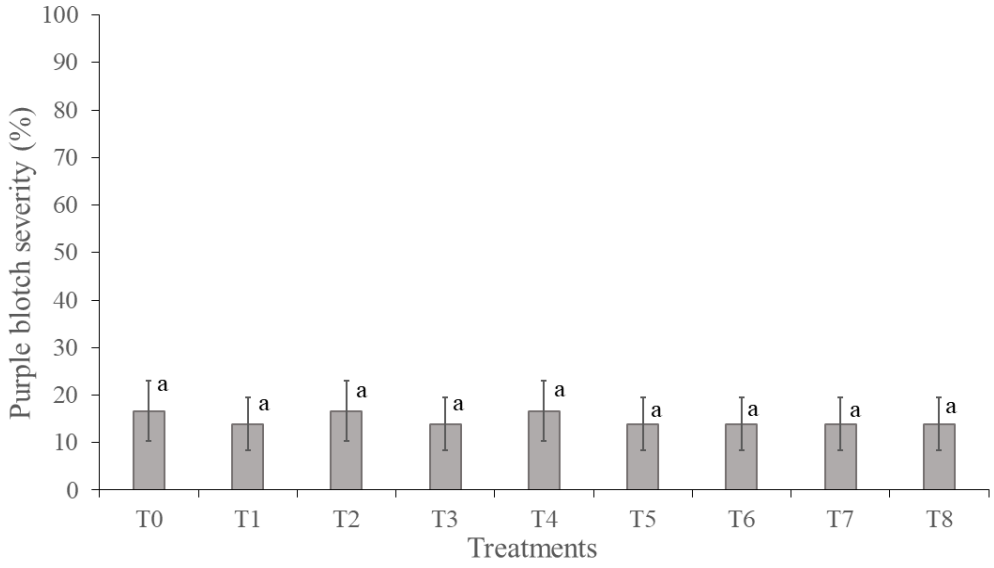

Fig. 3. Severity of onion purple spot disease at 100 days after transplanting

Bars are means ±SD. Bars with the same letter are not significantly different at p = 0.05 according to Duncan's Multiple Range Test. T0: control, T1: Soil treated with A. indica powder and leaves treated with aqueous extract of A. indica, T2: Soil treated with A. indica powder and leaves treated with Azoxystrobin, T3: soil treated with A. indica powder and leaves treated with Mancozeb, T4: Soil treated with L. multiflora powder and leaves treated with aqueous extract of L. multiflora, T5: soil treated with L. multiflora powder and leaves treated with Azoxystrobin, T6: soil treated with L. multiflora powder and leaves treated with Mancozeb, T7: leaves treated with Azoxystrobin, T8: leaves treated with Mancozeb.

3.1.5 BULB YIELD

Figure 4 shows the evolution of bulb yield depending on the treatments applied. Statistical analysis showed that there was a significant difference (p = 0.0001) between the treatments. The highest yields (41 to 43.25 t/ha) were recorded in the plots treated with *A. indica* powder (T1, T2, T3) and in those treated with powder powder and aqueous extract of *L. multiflora* (T4). The other treatments (T5, T6, T7) recorded yields similar to that of the control but higher than that of the Mancozeb treatment alone.

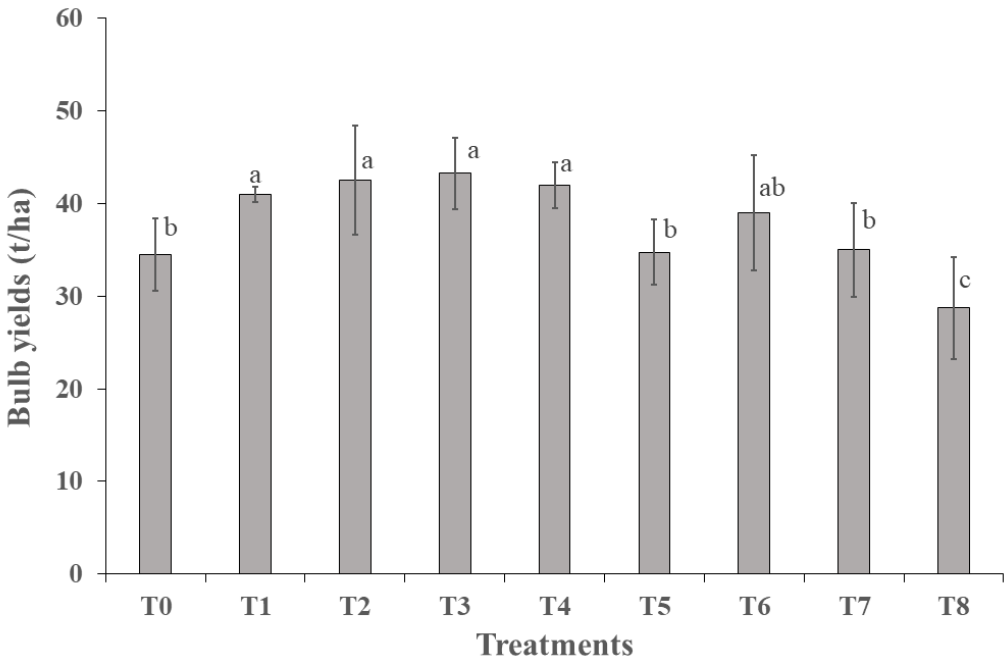

Fig. 4. Onion bulb yield according to treatments

Bars are means ± SD. Bars with the same letter are not significantly different at p = 0.05 according to Duncan's Multiple Range Test. T0: control, T1: Soil treated with A. indica powder and leaves treated with aqueous extract of A. indica, T2: Soil treated with A. indica powder and leaves treated with Azoxystrobin, T3: soil treated with A. indica powder and leaves treated with Mancozeb, T4: Soil treated with L. multiflora powder and leaves treated with aqueous extract of L. multiflora, T5: soil treated with L. multiflora powder and leaves treated with Azoxystrobin, T6: soil treated with L. multiflora powder and leaves treated with Mancozeb, T7: leaves treated with Azoxystrobin, T8: leaves treated with Mancozeb.

3.2 DISCUSSION

The *in vitro* experiment showed that aqueous extracts of *A. indica* and *L. multiflora* inhibited the growth of *A. porri,* as has already been reported by some authors against various phytopathogenic fungi. The efficacy of the aqueous extracts augmented proportionally to the doses, as confirmed by [21].

The antifungal properties of extracts of *Lippia* species have been demonstrated in previous studies. For example, [22] found that aqueous extract of *L. multiflora* inhibited germ tube growth of *Puccinia arachidis,* while [23] demonstrated that extracts of *Lippia javanica* and *Lippia rehmannii* significantly inhibited mycelial growth of *Penicillium digitatum*. The inhibition of fungal growth by the aqueous extract of L. multiflora can be explained by the fact that the leaves contain compounds with antifungal properties. In fact, phytochemical analyses performed by [24] and [19] showed that the leaves of *L. multiflora* contain a variety of phenolic compounds such as geniposide, nuomioside A, samioside, verbascoside, alyssonoside, isoverbascoside, isonuomioside and leucosceptoside. The antifungal properties of these phenolic compounds have been demonstrated by several authors [25], [19].

For *A. indica,* [26] showed that the aqueous leaf extract exhibited potent antibacterial and antifungal activity. [27] also reported that the aqueous seed extract inhibited the mycelial growth of *Sclerotium rolfsii* and leaf extracts inhibited the growth of *Alternaria solani*. The antibacterial and antifungal activity of the aqueous extract of *A. indica* leaves is thought to be due to the presence of high concentrations of azadirachtins, quercetin and β-sitosterol in the leaves, as shown by [28].

In the field, statistical analysis showed no significant differences between the severity of onion purple blotch disease in treated and control plots. This could be due to the low severity of the disease throughout the trial. Nevertheless, the treatments had a positive effect on onion growth and production. This could be explained by the fact that the decomposition of the powders in the soil can help to improve soil fertility. In this regard, [29] reported that *A. indica* derivatives improved soil fertility. Furthermore, according to [30], in addition to their nematocidal activity, *A. indica* triterpenoids inhibit nitrification, thereby increasing soil nitrogen availability.

4 CONCLUSION

This study aimed to evaluate the effectiveness of treatment combinations with fungicides and plant-based pesticides in the control of *A. porri,* onion growth and bulb production. The results of this study showed that the application of soil powders in combination with foliar treatments for the biocontrol of purple blotch disease improves onion growth and production and can provide an alternative to the overuse of synthetic fungicides.

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