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Integrated management of leaf spot of peanut with aqueous leaf extract of *Lippia multiflora* Moldenke and Chlorothalonil

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ABSTRACT

This study is a contribution in the fight against early and late leaf spots (two fungal diseases) of peanut (*Arachis hypogaea* L.). The objectives of the study was to evaluate the antifungal effect of integrated treatment which composed of an aqueous extract of *Lippia multiflora* Moldenke leaves with a chemical fungicide (Chlorothalonil), alternately. The experiment was conducted in Gampêla district in Burkina Faso during the crop season 2016-2017. From 30 days after sowing (DAS), two varieties of peanuts, TS32-1 and B106, were sprayed five times, every 15 days, with the leaf plant extract (50 g dried leaves/1 l water) combined or not with Chlorothalonil. Marked effects on leaf spots were noticed from the first spraying, limiting the defoliation rate, the necrotic leaf area, and increasing pod and seed yield. Treatment means were compared using Student Newman-Keul test. Alternative treatments with Chlorothalonil fungicide reduced significantly the severity of diseases in the field. The maximum disease effect was observed on the untreated plot reaching the value of 6 in sensitive variety TS32-1. The integrated treatments, T2, T3 and T4 limited the disease at level 4 up to 90 DAS at last treatment. Among the alternative treatments, the highest pod and seed yields were observed with T4 (1519 and 408 kg ha^{-1}) and T3 (1479 and 443 kg ha^{-1}). This work confirmed the possibility to reduce the number of applications of fungicide in field by integrated treatments using aqueous extract of *L. multiflora* Moldenke leaves against leaf spots control. Therefore, the application of these plants extracts and Chlorothalonil in the control of leafs pots could lead to substantial yield increases for producers whilst ensuring safer conditions for consumers, producers and the environment.

INTRODUCTION

Peanut or groundnut (*Arachis hypogaea* L.) is an important oil seed crop in the world. In 2014, a total area of 25,680,294 hectares for a total production of 42,444,356 tons and a yield of about 1,652 kg ha^{-1} was recorded (FAO of the United Nations, 2016). As a subsistence crop or cash crop, groundnut is widely used for food and feed. Its rusticity, plasticity, the multiplicity of uses makes this plant a highly appreciated oleoprotea-ginous crop (Schilling et al., 1996). However, groundnut remains a plant subjected to several parasitic attacks, particularly fungal, which causes sharp declines in yields.

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Among fungal diseases of groundnut, leaf spot (early and late leaf spots) is the major constraint in its production in Tropical Africa. The causative agents of these diseases are *Cercospora arachidicola* Hori. and *Phaeoisariopsis personata* (Berk and M. A. Cuortis) Vanarx. Leaf spots damage the plant by reducing the available photosynthetic area by lesion formation, damaging the photosynthetic apparatus of neighbouring, apparently healthy tissues and inducing early leaf abscission. Resistance to leaf spot is a valuable trait that affects yield potential of peanuts. The use of chemicals to control groundnut disease is considered to be the most effective strategy and the conventional way (Fravel et al., 2005; Kiran et al., 2006). But, the need for applications (4-5 sprays) of recommended fungicides, such as chrorothalonil, mancozeb, folicur, discouraged extensive adoption of groundnut by resource-poor farmers of the rain-fed production system (Kishore and Pande, 2005). Moreover, repeated use of synthetic pesticides can lead to induction of genetic resistance in targeted organisms as shown in the control of *Ustilago nuda*, the causal agent of wheat smut, bycarboxine (Leroux, 1987). Accordingly, there is a need to identify alternative methods of disease management that are both economical and eco-friendly.

In many instances, plant extracts have been used as effective and cheaper alternatives to chemicals in combatting insects, pests and fungal diseases of plants (Djibo, 2000; Nebie, 2006; Somda et al., 2007; Zidaet al., 2008; Koïtaet al., 2012). Some works were reported antifungal properties of aqueous extracts of *Allium sativum*, *Cymbopogon proximus*, *Carum carvi*, *Azadirachta indica* and *Eugenia caryophyllus* against *Fusarium oxysporum, Botrytis cinerea* and *Rhizoctonia solani* (Alkhail, 2005). Likewise, extracts of leaves of papaya tree (*Carica papaya* L.) and vernonia (*Vernonia amygdalina* Delile) were successfully tested *in vitro* against the pathogenic fungi of groundnut (Ogwulumba et al., 2008). Some rice cultivars exhibit antifungal activity against *Macrophomina phaseolina* and *Ascochyta rabiei* (Bajwa and Ramzan, 2004). Recently, Riaz et al. (2009) evaluated the efficacy of plant extracts of *Alstonias cholaris, A. indica*, *Lawsonia alba*, *Allium cepa*, *A. sativum* and *Zingber officinale* against fungal pathogen *F. oxysporum* f.sp. gladioli causing corm-rot disease of Gladiolus. Even, recent research revealed that aqueous extracts of *Agave sisalana* contained antifungal properties against fungal pathogens of rice (*Magnaporthe grisea*) (Kassankogno et al., 2015).

In spite of their advantage of plants extracts in controlling pests and diseases in agriculture, it must be recognized that most of these studies are carried out *in vitro* and application to the field often gives less potential than chemical fungicide. The aim of this research was to reduce the number of fungicide applications by alternate use with a plant extract.

MATERIALS AND METHODS

Study site and experimental design

Field evaluation of integrated application of plant extract and Chlorothalonil were conducted during 2016-2017 crop season at the Gampela District, localed at longitude 12.22°W and latitude 12.25°N of Ouagadougou, Burkina Faso. The annual temperature ranges between 21.5 and 42.8°C. The field soil shows a great diversity. This is among other ferruginous soils, waterlogged soils, ferruginous hydromorphic soils, the little evolved soils of anthropogenic input and eutrophic brown soils (Thiombiano and kampmann, 2010). The experiment was a randomised complete block design with three replicates per treatment. The field trial consisted of six treatments involving four integrated applications at different combinations (plant extract+Chlorothalonil) and two controls (fungicide and untreated).

The fungicide use is BALEAR 720 SC (Bravo Ultrex, 82.5 WDG, Syngenta Crop Production, Greensboro, NC; 2 kg ha^{-1}). The active ingredient is Chlorothalonil. The chemical formula of its active substance is $C_8Cl_4N_2$. BALEAR 720 SC has systemic properties. Its action is preventive. Six treatments were applied to six plots of the groundnut varieties (TS32-1 and B106). The plot size was 224 m^2. Row to row distance was 1 m and plant to plant distance was maintained 15 cm. A fertiliser composed of 14–23–14, Nitrogen-Phosphorus-Potassium (N-P-K) was applied at a rate of 100 kg ha^{-1}, 22 DAS in experimental repeats.

Plant material

The test was conducted on two groundnut varieties TS32-1 and B106. The TS32-1 variety resulted from a cross between the varieties spantex and TE3. TS32-1 is a Spanish-type groundnut recommended by the National Institute of Agricultural Research of Burkina Faso (INERA). As an early maturing (90 days) variety, it is widely cultivated in Burkina Faso. However, TS32-1 is highly susceptible to early and late leaf spots, rust, rosette and *Aspergillus flavus* (Subrahmanyam and Hildebrant, 1992).

B106 resulted from a cross between an American variety of Texas (1333) and Nama, the local variety of Burkina Faso. The variety B106 is a late maturing (105-110 days). This variety is moderately resistant to early and late leaf spots, rust, and is a Virginia-type groundnut.
L. multiflora leaves have been collected in Gampêla, a village located in the center of Burkina Faso. The plant can reach 3 to 5 m in height (Guinko and Assi, 1981). It has bluish leaves with a camphorous odor of friction. A botanical certification of the species has been made by the Plant Ecology and Biology Laboratory from the

University of Ouagadougou (Ouaga I).

Plant extract preparation

Fresh leaves of *L. multiflora* were collected from local fields in Gampêla district. The leaves collected have been dried in the shade, finely crushed and kept in a fresh and dry place, sheltered from light. The extraction has been carried out adding 1 l of distilled water to 50 g of powder followed by homogenization for 2 h and filtered through muslin cloth (5 µm). The resulting filtrate was collected and used for plot treatment.

Field treatments applied

The following integrated treatments applied as a foliar spray at 30, 45, 60, 75 and 90 DAS were evaluated for leaf spot control in field:

- T1: untreated plot;
- T2: Chlorothalonil one time (30 DAS), plant extract four times (45, 60, 75, 90 DAS);
- T3: Chlorothalonil two times (30, 45 DAS), plant extract three times (60, 75, 90 DAS);
- T4: Chlorothalonil three times (30, 45, 60 DAS), plant extract two times (75, 90 DAS);
- T5: plant extract alone (30,45, 60, 75, 90 DAS);
- T6: Chlorothalonil alone (30, 45, 60, 75, 90 DAS).

Plants were sprayed with aqueous extracts or fungicide using a hand-held Solo branded sprayer.

Data collection and statistical analysis

Quantification of disease severity at 15 day intervals, starting at 30 DAS until harvest, was determined using the ICRISAT scale, which ranges from 1 to 9 (Subrahmanyam et al., 1995). The disease score depends on visual estimate of necrosis and defoliation. A disease rating of 1 means no disease and a rating of 2 means lesions present on lower leaves with no defoliation. Ratings of 3 to 8 are associated with increasing levels of defoliation and necrosis. A rating of 9 implies defoliation of almost all leaves leaving bare stems, with any leaflets present having many leaf spots. The ICRISAT leaf spot disease rating was converted to percent necrosis (Equation 1) and to percent defoliation (Equation 2) (Singh et al., 2013) at 75 DAS and 90 DAS.

$$\text{Necrosis}\ (\%) = 1.36 \times \frac{10}{9} \times \text{IS} - 1.45 \quad (1)$$

$$\text{Defoliation}\ (\%) = 12.5 \times \text{IS} - 12.5 \quad (2)$$

Where, IS represents ICRISAT visual score.

After harvest, the pods were sun-dried and pod yield was calculated per hectare. To ensure homogeneity of variances and normality of the distribution of each variable, data recorded as percentages were arcsine-transformed. Analysis was done using one-way analysis of variance (ANOVA) and means were separated at 5% level of probability. Treatment means were compared using Student Newman-Keul test (5%). Statistical analyses were done with the software XLSTAT-2010.

RESULTS

Disease development

The disease development was assessed during the five notations dates on sensitive variety, TS 32-1 (Figure 1). The difference between treatments began to be seen from 60 DAS. From this date, integrated treatments and the fungicide alone better limited the disease evolution than T1, untreated plod. The maximum disease effect was observed on this plot reaching the value of 6. The integrated treatments, T2, T3 and T4 limited the disease at level 4 up to 90 DAS. *L. multiflora* aqueous leaf extract alone (T5 plot) and the fungicide alone (T6 plot) provided the best control of the disease with a mean score of 3.33 and 3, respectively.

For the tolerant variety, B106, data on disease evolution at the different dates of notation are presented in Figure 2. The highest disease development is observed in the control plot, reaching the value of 3 after 90 DAS. Integrated treatments as well as fungicide alone induced a slower evolution with a maximal score of 2.33. The slowest evolution was noticed at T4 plot with a score of 2; only small necrotic spots were observed on basal leaves at 90 DAS.

The effect on disease control is similar when the fungicide applied once against three applications of plant extract (T2) and also when it applied twice against two applications of plant extract (T3) at 90 DAS. The plant extract used alone (T5) showed a more rapid evolution of the disease at 60 and 75 DAS, but the level was maintained for the fungicide alone (T6) at 90 DAS.

Percent necrosis and defoliation

Disease evolution was assessed by percent necrosis and defoliation at 75 and 90 DAS (Table 1). At 75 DAS, the highest percent necrosis for TS32-1 was observed with T1, with the negative control having a value of 5.09%. The lowest percent necrosis of 1.57% was observed with T6, in the fungicide alone plot. All the integrated treatments gave similar values of 2.57%. On the same

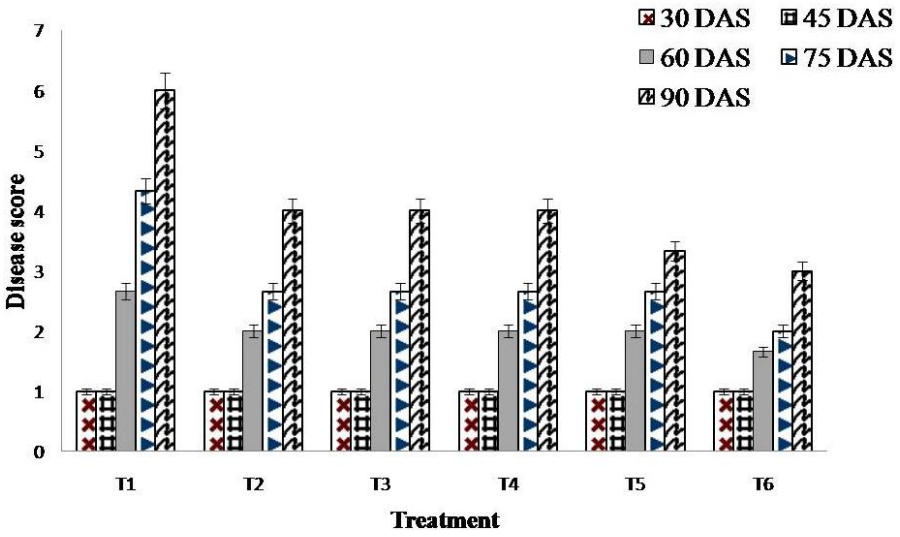

Figure 1. Effect of *L. multiflora* aqueous leaf extract alone or combined with Chlorothalonil application on peanut sensitive variety TS 32-1for control of leaf spot.
Caption: Treatments were applied as foliar sprays at 30, 45, 60, 75, and 90 days after sowing. Disease score was measured on a 1-to-9 rating scale. **T1,** Untreated plot (negative control); **T2,** Chlorothalonil one time (30 DAS), plant extract four times (45, 60, 75, 90 DAS); **T3,** Chlorothalonil two times (30, 45 DAS), plant extract three times (60, 75, 90 DAS); **T4,** Chlorothalonil three times (30, 45, 60 DAS), plant extract two times (75, 90 DAS); **T5,** *L. multiflora* aqueous leaf extract alone e; T6: Chlorothalonil alone. Each data point is an average of three replications. Standard errors are shown by vertical bars.

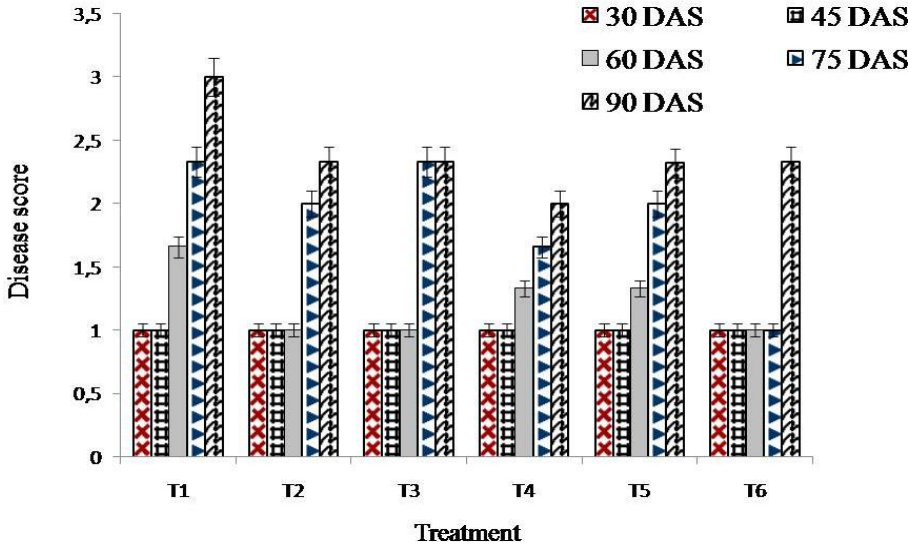

Figure 2. Effect of *L. multiflora* aqueous leaf extract alone or integrated with Chlorothalonil application on peanut resistant variety (B106) for control of leaf spot.
Caption: Treatments were applied as foliar sprays at 30, 45, 60, 75, and 90 days after sowing. Disease score was measured on a 1-to-9 rating scale. **T1,** Untreated plot (negative control); **T2,** Chlorothalonil one time (30 DAS), plant extract four times (45, 60, 75, 90 DAS); **T3,** Chlorothalonil two times (30, 45 DAS), plant extract three times (60, 75, 90 DAS); **T4,** Chlorothalonil three times (30, 45, 60 DAS), plant extract two times (75, 90 DAS); **T5,** *L. multiflora* aqueous leaf extract alone; **T6,** Chlorothalonil alone. Each data point is an average of three replications. Standard errors are shown by vertical bars.

Table 1. Percentage necrosis and defoliation rate caused by leaf spot at 75 and 90 DAS in integrated treatments plots and control.

Variety	Treatment	Percent necrosis (%)		Defoliation rate (%)	
		75 DAS	90 DAS	75 DAS	90 DAS
TS32-1	T1	5.09a	7,62a	41.67a	62.5a
	T2	2.57b	4,6b	20.83b	37.5b
	T3	2.57b	4,6b	20.83b	37.5b
	T4	2.57b	4,6b	20.83b	37.5b
	T5	2.57b	3,59c	20.83b	29.17c
	T6	1.57c	3,08cd	12.5c	25.0c
B106	T1	1.57a	3,08a	12.5a	25.0a
	T2	1.06ab	2,07b	8.33b	20.83b
	T3	1.57a	2,07b	12.5a	20.83b
	T4	1.06ab	1,57bc	8.33b	20.83b
	T5	1.57a	2.07b	12.5a	20.83b
	T6	0.06b	1,57bc	0.00c	12.5c

T1, Untreated plot (negative control); **T2,** Chlorothalonil one time (30 DAS), plant extract four times (45, 60, 75, 90 DAS); **T3,** Chlorothalonil two times (30, 45 DAS), plant extract three times (60, 75, 90 DAS); **T4,** Chlorothalonil three times (30, 45, 60 DAS), plant extract two times (75, 90 DAS); **T5,** plant extract alone; **T6,** Chlorothalonil alone.

date, on the tolerant variety B106, the percentage necrosis was lower with values of 1.06 (T2 and T4) and 1.57% (T1, T3 and T5), and no differences among these treatments were observed. At 90 DAS, the percentage necrosis has evolved.

Thus for the TS 32-1 variety, the integrated treatments have increased from 2.57 to 4.6%; a less significant change than untreated plot, whose percentage necrosis increased to 7.62%. A slight evolution of the necrotic spots is also observed in Chlorothalonil alone plot who has showed a necrosis percentage of 3.08%.

T5 is the only treatment statistically equivalent to fungicide alone with a value of 3.59%. For B106, the trend observed is the same. The necrotic spots increased between the two dates of notations. Nevertheless, they remain less important than those observed in TS32-1. T1 showed the highest necrotic evolution of 3.08 at 90 DAS. Integrated treatments (T2, T3 and T4) and Chlorothalonil alone showed statistically equivalent values.

Parallel to the percent necrosis, the defoliation rate was considerably higher in TS 32-1 than in B106 (Table 1). The highest defoliation was recorded on untreated plot 1, which increased from 41.67% at 75 DAS to 62.5% at 90 DAS. On this variety, integrated treatments (T2, T3 and T4) gave similar rates, that is, 20.83% at 75 DAS and 37.5% at 90 DAS. At 90 DAS in TS32-1, the fungicide alone treatment (T6) and plant extract alone (T5) recorded equivalent values. On the same date, 25% defoliation was recorded on the B106 negative control, T1. The integrated treatments displayed the same values (20.83%). T6 with a defoliation rate of 75 DAS was increased by 12.5% at 90 DAS.

Pod and seed yields

Pod and seed yields of integrated treatments were compared to Chlorothalonil (T6) and negative control T1 (Table 2). With susceptible variety, TS 32-1, the highest pod yields were observed for T3, T4 and T6 with yields of 1479, 1519 and 1618 kg ha^{-1}, respectively, with no difference ($P > 0.05$) among those three treatments. T2 displayed a different value greater than T1 and T5, that is, 1362 kg ha^{-1}. On the other hand, T5 treatment recorded the nearest values from untreated plot T1 in pod yield with yields of 1162 and 1005 kg ha^{-1}, respectively. T2 displayed a different value greater than T1 and T5, that is, 1362 kg ha^{-1}.

For B106, the highest pod yield was observed for T4 with yield of 1246 kg ha^{-1}. This was followed by T6 and T3 with pod of 1143 and 1142 kg ha^{-1}, respectively. Treatments that recorded values less than 1000 kg ha^{-1} were T2 and T5 with yields of 961 and 930 kg ha^{-1}, respectively. The lowest pod yield of 850 kg ha^{-1} was observed in T1, the negative control to B106. All these data indicated that reducing the number of fungicide applications to only two times (T3) would give the same result if applied six times (T6). Leaf spot resistance probably contributed to a better seed fill and higher seed filling rate, which may have resulted in the higher yield

Table 2. Pod and seed yields in experimental field regarding applied treatments (integrated treatments, plant extract alone, Chlorothalonil alone and control).

Variety	Treatment	Pod yield (Kg ha^{-1})	Seed yield (Kg ha^{-1})
TS32-1	T6	1618,99a	485,70a
	T4	1519,77a	408,75a
	T3	1479,07a	443,72a
	T2	1362,51b	455,93a
	T5	1162,48c	348,74b
	T1	1005,32d	331,60b
B106	T6	1246,17a	373,85a
	T4	1143,93b	342,88a
	T3	1142,93b	288,34b
	T2	961,12c	292,19b
	T5	930,21c	279,06b
	T1	850,58d	255,17b

T1, Untreated plot (negative control); **T2,** Chlorothalonil one time (30 DAS), plant extract four time (45, 60, 75, 90 DAS); **T3,** Chlorothalonil two time (30, 45 DAS), plant extract three time (60, 75, 90 DAS); **T4,** Chlorothalonil three time (30, 45, 60 DAS), plant extract two time (75, 90 DAS); **T5,** plant extract alone; **T6,** Chlorothalonil alone.

observed for these.

Seed yield generally followed the same trend as pod yield for both variety (Table 2). Seed yield of TS32-1 for T6, T4, T3 and T2 are similar with seed yields of 485, 408, 443 and 455 kg ha^{-1}, respectively. For the tolerant variety, B106, T4 with 342 kg ha^{-1} seed yield is identical to the fungicide alone T6, having 373 kg ha^{-1}. There were no significant differences in the other treatments (T2, T3, and T4) having values of 288, 292 and 279 kg ha^{-1} respectively.

DISCUSSION

The efficacy of foliar sprays with *L. multiflora* aqueous leaf extract alone or combined with Chlorothalonil in reducing leaf spot of peanut was investigated. It is necessary to control the severity of this disease in the field in order to ensure a good yield. In the recent years, there has been a great deal of effort to reduce the risk of pesticides and chemicals (Dubey et al., 2011). Thus, the aqueous extract of the leaves of *L. multiflora* Moldenke and the integrated treatments with the fungicide expressed an antifungal activity against the fungi tested both in the course of the disease, and in the pod and seed yields. The effect of fungicide alone on the pod yield and seed yield was similar to integrated treatments [Chlorothalonil + plant extract (T3 and T4)] with sensitive variety. The negative control recorded the highest values in leaf spot and the lowest in the yield. Although superior to negative control, the plant extract alone did not record performance equivalent to the pesticide alone and integrated treatments. But the treatment showed efficacy in controlling the disease. Other previous studies have confirmed the *in vitro* antifungal efficacy of *L. multiflora* on the fungi tested by inhibiting their germination (Koïta et al., 2012). Studies on *L. rehmannii* indicated a real efficiency of essential oil against *R. solani*, *F. oxysporum* and *Penicillium digitatum* fungi, which are respectively potato, maize and orange tree pathogens (Linde et al., 2010). Recent work has shown that the activity of this aqueous extract induces a control of the disease in the field and an increase of the yield to more than 10% compared to the negative control (Koïta et al., 2017). The integrated use of this plant with fungicide resulted in better control of the disease and a substantial gain in yield. Alternative treatments (plant extract / chemical fungicide) were tested with other plant species. Thus, integrated application of *Datura metel* leaf extract and Chlorothalonil were evaluated for improved late leaf spot and rust control in the field (Kishore and Pande 2005). Integrated application of plant extract and fungicides or biocontrol agents have been successful in improving control of anthracnose, and pod blight of soybean (Chandrasekaran and Rajappan, 2002) and sclerotium rot of potato (Solunke et al., 2001).

Thembo et al. (2010) highlighted a strong antifungal activity of extracts of *Tagetes minuta*, *Lippia javanica* and *Vigna unguiculata*. They showed that these plant species extracted with dichloromethane are effective against *Fusarium verticillioides* and *Fusarium proliferatum in vitro*.

Conclusion

Integrated treatments of *L. multiflora* extract and fungicide showed good efficacy in inhibiting the development of leaf spot in the field. Symptoms of phytotoxicity or stunting of growth were not observed in any of the treated plants. One might think of a synergy of action between fungicide and extract to reduce leaf spot evolution in natural conditions. The scores obtained at 90 JAS show that there was no significant difference between the integrated treatments and the fungicidal treatment alone. This shows that the number of times the chemical could be used in real-life treatments could be reduced. This will help protect humans and the environment from chemical pollution. The alternate use of *L. multiflora* extracts with Chlorothalonil is reported for the first time in this study. It is a proof that the reduction of fungicide in agriculture is possible. These results could be used as main component in integrated pest management program. In further studies, it would also be wise to test this program on pathogens in other major crops using pesticides, such as, rice and maize. To improve the performance observed, we could consider extending the experiment by increasing the dose of the plant aqueous extract or by reversing the treatment schedule by starting with the applications of the plant extract and ending with the fungicide.

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