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Methanolic extracts of three weeds as botanical fungicides to control peanut rust disease

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Abstract. *Yusnawan E, Inayati A. 2016. Methanolic extracts of three weeds as botanical fungicides to control peanut rust disease. Nusantara Bioscience 8*: *117-122*. Rust disease caused by *Puccinia arachidis* is one of the most important diseases on peanuts. Ageratum (*Ageratum conyzoides* L.), spiny amaranth (*Amaranthus spinosus* L.), and coco-grass (*Cyperus rotundus* L.) had the ability to inhibit plant pathogens. The aim of the research was to obtain the weed extracts effective to control peanut rust disease. The three weeds were ground to obtain fine particles, macerated in methanol for 18 h and evaporated the solvent using vacuum rotary evaporator. Methanolic crude extracts obtained from ageratum, coco-grass, and spiny amaranth at concentrations of 0.1%, 1.0%, 2.5%, and 5.0% were applied to the uredospores and peanut plants infected with the pathogen. Applications of 5% ageratum and 5% coco-grass extracts suppressed the spore germinations by 78 to 80% and 76 to 80%, respectively. Disease intensities on Kancil cultivar treated with 5% crude extract of ageratum were 18 to 22% and 29 to 31% after three and four times of extract applications. Disease intensities on untreated plants were 30 to 32% and 43 to 46%, whereas the chemical application suppressed the disease intensities up to 16 to 18% and 15 to 17% at the same time of observation. Preliminary phytochemical screenings showed that the methanolic extract of ageratum contained alkaloids, flavonoids, tannins, saponins, and terpenoids. Crude extract of 5% ageratum was effective to control rust disease on peanuts, therefore, could be used as an alternative control agent to suppress the disease.

Keywords: *Ageratum conyzoides*, *Amaranthus spinosus*, botanical fungicide, *Cyperus rotundus*, peanut rust disease

INTRODUCTION

Peanut rust disease caused by *Puccinia arachidis* Speg. is one of the most important foliar diseases on peanut with almost worldwide distribution. The presence of orange-colored pustules is the specific symptom of this disease which appears on the lower surface of the leaves. Rupture pustules release masses of reddish-brown spores. This disease reduces the pod yield from 6 to 57% depending on the susceptibility of the genotypes (Subrahmanyam and McDonald 1987; Subrahmanyam et al. 2012). Control of this disease using chemical fungicides has been proven effective to reduce the infection, however, is not considered environmentally friendly. Applications of botanical fungicides could be one of the alternative controls which gave less negative impacts to the environment (Mushatq et al. 2012). Natural products extracted from plants have been used as antimicrobial agents and pesticides, because of their bioactive compounds, such as flavonoids, alkaloids, terpenoids, saponins, and tannins (Harborne 1998; Kamboj and Saluja 2008; Patil et al. 2010).

Ageratum conyzoides L. (ageratum, goatweed), *Amaranthus spinosus* L. (spiny amaranth) and *Cyperus rotundus* L. (coco-grass) have been known as weeds because of their invasive growth. Ageratum grew to dominate cultivated areas due to its ability to produce allelochemical compounds (Singh et al. 2003; Kong et al. 2004a,b, 2006). Besides having negative effects on the cultivated crops, this weed contained secondary metabolites which suppressed the growth of some pathogens (Kong et al. 2004c; Kamboj and Saluja 2008). Crude extract of goatweed inhibited the growth of both bacteria and fungi such as *Staphylococcus aureus*, *Candida albicans*, and *Cryptococcus neoformans* (Okunade 2002; Kong et al. 2004c; Dayie et al. 2008). In some African countries, this weed also had long been used to cure several diseases such as skin diseases, to cure wounds, and to treat infected diseases caused by bacteria (Kamboj and Saluja 2008).

Active compounds of spiny amaranth had been used as anti-inflammation, anti-malaria, anti-bacteria, anti diuretic, and anti-virus. Reports of *A. spinosus* as antimicrobial agents were still limited. Maiyo et al. (2010) reported that *n*-hexane fractions had anti bacterial properties against *Salmonella typhi*. Another species of *Amaranthus*, i.e., *A. viridis* or green amaranth inhibited 20-40% of mycelial growth of major seed-borne fungi including *Aspergillus niger*, *Alternaria alternata*, *Drechslera biseptata*, and *Fusarium solani* when chloroform extract of the leaves was tested *in vitro* (Mushatq et al. 2012).

Coco-grass had been reported to have antimicrobial properties against bacteria and fungi (Parekh and Chanda 2006; Bisht et al. 2011; Singh et al. 2011). Essential oil obtained from roots of this weed inhibited the growth of *Bacillus subtilis*, *Escherichia coli*, *S. aureus*, and *Pseudomonas aeruginosa* at the concentration of 100% (Bisht et al. 2011), however, high concentration of the oil used appeared not particularly effective for large

application. Antifungal properties had also been reported against *Aspergillus fumigatus* and *Candida parapsilosis* at the concentrations of 25% and 50%, respectively. In addition, the application of 25% also inhibited spore productions of *Aspergillus flavus* and *Fusarium oxysporum*. Ethanolic and water extracts inhibited several pathogenic bacteria such as *B. cereus, B. subtilis, E. coli, P. aeruginosa,* and *S. aureus* (Parekh and Chanda 2006). An *in vitro* study conducted by Singh et al. (2011) showed that ethyl acetate extract of the roots was effective to suppress the growth of *Alternaria, Colletotrichum, Curvularia,* and *Helminthosporium*. The use of weed extracts to control plant pathogens could be promising. Therefore, this study aimed to determine the effectiveness of ageratum, spiny amaranth and coco-grass extracts to control peanut rust disease. A preliminary phytochemical screening test was conducted to determine the active compounds in the three weed extracts of Indonesian origin.

MATERIALS AND METHODS

Sample preparation and extraction

Ageratum, coco-grass, and spiny amaranth were collected from around Kendalpayak Research Station, Indonesian Legumes and Tuber Crops Research Institute (ILETRI), Malang, East Java. The three weeds were air dried (moisture content ±10%) and crushed into fine particles of 80 mesh. The fine particles were macerated in methanol (1:10 w v^{-1}) in three separate volumetric flasks. After shaking for 4 h at 100 rpm, the suspension was incubated for 18 h at room temperature. The supernatant was separated from the pellet by centrifugation at 300 rpm for 15 min. The supernatant containing methanol was evaporated with vacuum rotary evaporator to obtain about 10% (w w^{-1}) concentrated extract from the initial weed weight. During vacuum evaporation, the temperature was maintained at 50ºC. The concentrated extract obtained from evaporation was placed in a desiccator for 24 h and stored at 4ºC before used in amber bottles.

Spore germination tests

Treatments were arranged in a completely randomized design with two factors in triplicates. The first factor was three weed extracts (ageratum, coco-grass, and spiny amaranth) and the second factor was the concentration of the weed extracts (0.1%, 1.0%, 2.5%, and 5.0%, respectively). Uredospores germinated in phosphate buffer saline were used as a control. Uredospores of *P. arachidis* were collected from 10-week infected peanut plants from an ILETRI greenhouse. The infected leaves were incubated for 2 days in Petri dishes with wet cotton layers to maintain humidity at around 98%. The uredospores were collected from the leaves and suspended into sterile water containing 0.05% Tween 20. The concentrations of 0.1%, 1.0%, 2.5%, and 5.0% of each extract were tested to the uredospores in test tubes. Then numbers of germinated, ungerminated and lysed uredospores were counted after 24 h of the treatments. Germinated spores were characterized by the elongation of the germ tubes (Yusnawan 2015).

Greenhouse experiment

Treatments were arranged in a two factor of completely randomized block design in triplicates. The first factor was the three weed extracts (ageratum, spiny amaranth, and coco-grass), the second factor was four concentration levels of the weed extracts (0.1%, 1.0%, 2.5%, and 5.0%). Negative control was the application of water, whereas a positive control was the application of 0.1% of difenoconazole fungicide according to the recommended dosage. Peanut seeds of Kancil cultivar were sown two seeds per hole; each replicate consisted of three pots. Inoculation with uredospores was conducted at three weeks after planting (WAP) using a hand sprayer, with spraying volume equal to 400 L ha^{-1} and repeated at 4 WAP to ensure that the infection occurred, as indicated by brown-yellow rust on the adaxial of the leaves. The applications of the weed extracts were conducted at 5, 6, 7, 8, and 9 WAP at 4 pm. Disease intensity, number of pustules per cm^2 and accumulation of pustules per cm^2 were recorded at 8 and 9 WAP or after applications of the third and the fourth of the weed extracts. Percentage of infected plants represented disease intensity was measured based on the scoring according to Subrahmanyam et al. (1995). Pod yield was also recorded after harvesting.

Phytochemical screening tests

The screening tests were conducted using these chemicals: alkaloids were detected with Mayer and Wagner reagents, flavonoids were reacted with Mg and HCl, tannins were tested with gelatin and $FeCl_3$, terpenoids with Liebermann-Burchard method and H_2SO_4, and saponins were determined with its ability to form stable foam (Harborne 1998). The color changes or ability to form sedimentation after the weed extracts were reacted with suitable reagents indicating the presence of certain compounds. Extracts contained alkaloids and tannins if sedimentation occurred after being reacted with the suitable reagents. The change of the color in the extracts after reaction indicated the presence of flavonoids, tannins, and terpenoids.

Thin Layer Chromatography (TLC)

Silica gel F_{254} plates were used to separate compounds based on the compound polarities. A 5 µl extract was spotted on the silica gel plates and the plates were left to dry. Elution was carried out to separate the active compounds. The mobile phases were methanol: chloroform (0.5:9.5 v v^{-1}) to separate alkaloid compounds (Wagner and Bladt 1996), chloroform: methanol (9:1 v v^{-1}) to separate flavonoids (Harborne 1998), *n*-hexane: acetone (4:1 v v^{-1}) to separate saponins (Marliana and Suryanti 2005), and *n*-hexane: ethyl acetate (2:8 v v^{-1}) to separate triterpenoids (Wagner and Bladt 1996).

RESULTS AND DISCUSSION

Spore germination percentage

The spore germination test showed that of the 100 spores tested, the treatments of 2.5% and 5.0% ageratum

extracts inhibited 76-77% and 78-80% spore germination (Table 1). No difference in inhibition of spore germination was achieved when 2.5% and 5.0% coco-grass extracts were applied. Interestingly, more lysed spores as indicated by the breakdown of the spore wall were observed in the application of 5.0% spiny amaranth extract. Both ungerminated and lysed spores have used an indicator of the effectiveness of crude extract applications. In nature, only germinated spore will continue its life cycle to form mycelia which infect the healthy peanut plants.

Greenhouse experiment

The disease intensity due to the application of 5.0% ageratum extract was the lowest at the third application at 18.9 to 21.6% compared to that of the two weed extract treatments (Table 2). The application of this weed concentration showed the most effective control compared to that of the two other weed extracts at the same concentration. However, the application of 2.5% ageratum showed the same result as untreated crops (the application with water), which was not observed in spore germination test during *in vitro* study. The application with difenoconazole was able to suppress the disease intensity up to 18%.

At the fourth week after application, the disease intensity of the 5.0% ageratum extract application increased slightly to around 30% (Table 3), the lowest of all the applications. The extract of 5.0% spiny amaranth suppressed the disease intensity similar to that of 2.5% ageratum extract, which could also be considered for large field application. Unlike the applications of the weed extracts, the treatment using the fungicide showed no increase in disease intensity.

The numbers of pustules and the accumulation of the pustule numbers per cm^2 were recorded considering that the pustules as groups of uredospore were effective agents to infect the healthy plants in the field as long as supported by favorable environment, i.e. high temperature and high humidity. Low numbers in pustules will reduce the risk of the infection on healthy crops. The number of the pustules and the accumulation of the pustule numbers at 5.0% ageratum application were the lowest at the third week after application (6 to 8 spores and 12 to 14 spores) (Table 2) compared to the applications of spiny amaranth and coco-grass at the same concentration. Different results were observed in the fourth week after application. The number of pustules and the accumulation of pustule numbers on the infected leaves showed relatively the same results (Table 3).

Pod yield

Total peanut pod harvested from treated crops with crude extracts of the three weeds, difenoconazole and control varied from 9.9 to 47.6 g per plant (Figure 1). Fungicide application was the highest (47.6 g per plant) followed by the application of 5.0% and 2.5% ageratum extracts (33.4 and 27.5 g per plant). The same trend was observed at filled pods. Empty and immature pods yielded from treated crops with all crude extracts were not significantly different. Among weed extracts tested, the application of 5.0% ageratum extract saved 62% total pods and 64% filled pods compared to the untreated crop.

Phytochemical screening tests

This step was carried out to determine the active compound groups in each weed extract. The three weed extracts contained alkaloids, tannins, flavonoids, terpenoids, and saponins with different color intensities (Table 4). The phytochemical test of ageratum and coco-grass extracts reacted positively with all reagents, whereas spiny amaranth extract reacted negatively with the Mayer reagent on the alkaloid test and $FeCl_3$ on the tannin tests. Overall, the ageratum extract contained similar secondary metabolite groups as those of the coco-grass extract which may be responsible for suppressing peanut rust disease.

Table 1. Germination of uredospore of *P. arachidis* after the applications of *A. conyzoides*, *A. spinosus*, and *C. rotundus* methanolic extracts

Weed extract	Concentration Level (%)	Number of uredospore		
		Germinate	Ungerminate	Lyses
A. conyzoides	0.1	37.0 ± 3.0 e	57.0 ± 3.0 c	6.0 ± 0.0 e
	1.0	32.0 ± 1.0 f	58.7 ± 1.5 c	9.0 ± 1.0 d
	2.5	14.3 ± 1.5 h	76.3 ± 0.6 a	9.3 ± 2.1 d
	5.0	8.3 ± 0.6 i	79.3 ± 1.2 a	12.3 ± 0.6 b
A. spinosus	0.1	51.3 ± 1.2 b	42.7 ± 1.2 e	6.0 ± 0.0 e
	1.0	46.3 ± 0.6 c	43.3 ± 1.2 e	10.3 ± 1.5 cd
	2.5	41.0 ± 0.0 d	47.0 ± 1.0 d	12.0 ± 1.0 bc
	5.0	25.7 ± 2.1 g	57.3 ± 3.8 c	17.0 ± 2.0 a
C. rotundus	0.1	31.3 ± 3.5 f	66.0 ± 4.0 b	2.7 ± 0.6 f
	1.0	25.0 ± 3.0 g	69.0 ± 4.0 b	6.0 ± 1.0 e
	2.5	13.0 ± 1.0 h	77.7 ± 2.1 a	9.3 ± 1.2 d
	5.0	9.3 ± 2.5 i	78.0 ± 2.0 a	12.7 ± 0.6 b
Control		91.3 ± 2.1 a	8.7 ± 2.1 f	0.0 ± 0.0 g

Note: Numbers followed by the same letter in the same columns were not significantly different based on the LSD (α = 5%)

Table 2. Disease intensity (DI), number of pustule (Σ P), and accumulation of pustule number per cm^2 (Σ PA) on Kancil cultivar after the third application of methanolic weed extracts in a greenhouse

Weed extract	Concentration (%)	Disease intensity and pustule		
		DI (%)	Σ P	Σ PA
A. conyzoides	0.1	27.8 ± 1.0 b	7.9 ± 3.2 de	14.2 ± 4.7 de
	1.0	29.2 ± 2.1 ab	9.7 ± 3.7 cde	15.2 ± 3.1 cde
	2.5	29.1 ± 1.2 ab	6.4 ± 0.5 ef	12.8 ± 1.5 ef
	5.0	24.9 ± 6.6 c	6.7 ± 0.9 ef	12.7 ± 0.9 ef
A. spinosus	0.1	29.5 ± 1.7 ab	13.0 ± 1.9 abc	18.7 ± 0.9 abc
	1.0	29.8 ± 1.2 ab	14.6 ± 1.8 a	19.7 ± 2.5 ab
	2.5	29.8 ± 1.2 ab	12.2 ± 3.7 abc	19.4 ± 4.0 ab
	5.0	30.6 ± 0.2 a	10.9 ± 1.3 bcd	18.8 ± 3.3 abc
C. rotundus	0.1	29.3 ± 2.2 ab	13.7 ± 3.1 ab	18.8 ± 2.3 abc
	1.0	30.2 ± 0.5 ab	13.0 ± 2.2 abc	20.1 ± 2.4 ab
	2.5	30.1 ± 0.8 ab	10.6 ± 1.9 bcd	16.9 ± 3.1 bcd
	5.0	29.7 ± 1.5 ab	11.4 ± 1.3 abc	19.9 ± 3.0 ab
Water		30.8 ± 1.0 a	14.3 ± 2.3 a	26.6 ± 3.8 a
Fungicide		16.8 ± 1.0 d	3.7 ± 1.2 f	8.9 ± 1.1 f

Note: DI = disease intensity, Σ P = number of pustule, Σ PA = accumulation of pustule number. Numbers followed by the same letter in the same columns were not significantly different based on the LSD (α = 5%)

Table 3. Disease intensity (DI), number of pustule per cm^2 (Σ P), and accumulation of pustule number per cm^2 on Kancil cultivar after the fourth application of weed extracts in a greenhouse.

Weed extract	Concentration (%)	Disease intensity and pustule		
		DI (%)	Σ P	Σ PA
A. conyzoides	0.1	43.1 ± 0.7 a	11.2 ± 4.3 bcd	25.4 ± 1.9 def
	1.0	41.4 ± 2.3 ab	14.2 ± 1.7 abc	29.4 ± 4.5 bcde
	2.5	37.4 ± 2.3 cd	10.7 ± 1.2 cd	23.4 ± 1.7 ef
	5.0	29.9 ± 0.9 e	9.1 ± 1.9 d	21.8 ± 1.3 f
A. spinosus	0.1	43.7 ± 1.6 a	11.6 ± 3.3 bcd	30.2 ± 4.1 abcde
	1.0	43.7 ± 1.6 a	13.3 ± 1.2 abc	33.0 ± 1.7 abc
	2.5	43.7 ± 0.8 a	15.8 ± 2.7 a	35.2 ± 4.7 ab
	5.0	36.3 ± 4.3 d	9.1 ± 2.0 d	27.9 ± 5.0 cdef
C. rotundus	0.1	43.2 ± 0.8 a	11.7 ± 2.6 bcd	30.4 ± 1.1 abcd
	1.0	43.7 ± 1.6 a	13.9 ± 3.7 abc	34.0 ± 1.5 abc
	2.5	43.7 ± 0.8 a	15.8 ± 4.2 a	32.7 ± 6.0 abc
	5.0	40.1 ± 2.3 bc	8.1 ± 1.7 d	28.0 ± 2.8 cdef
Water		44.2 ± 1.4 a	14.3 ± 3.8 ab	36.3 ± 7.2 a
Fungicide		16.2 ± 0.9 f	2.8 ± 0.2 e	9.9 ± 1.5 g

Note: DI = disease intensity, Σ P = number of pustule, Σ PA = accumulation of pustule number. Numbers followed by the same letter in the same columns were not significantly different based on the LSD (α = 5%)

Thin layer chromatography

The TLC procedure was conducted to confirm the results obtained from the phytochemical screening test. The three extracts all contained alkaloids, flavonoids, terpenoids, and saponins. The number of spots varied in each extract as shown in Table 5. Flavonoids in the ageratum extract showed the highest number of the spots. Tannins were not well separated by the mobile phase of mixed glacial acetic acid: water: concentrated HCl (30:10:3 v v^{-1} v^{-1}) and methanol: water (1:1 v v^{-1}) (Harborne 1998). This mobile phase may not be able to separate the compounds or the polarity level of the compounds in the extract was too similar to the stationary phase. This caused the extract compounds more retained in the stationary phase.

Methanol and other alcoholic solvents were generally employed to extract plant secondary metabolites. These solvents were able to increase the cell permeability and to penetrate inside the cells, therefore, extracting more endocellular secondary metabolites compared to solvents with low polarity index such as *n*-hexane (Cannell 1998; Seidel 2012). Methanol with high polarity index was able to extract the secondary metabolites with polar properties, i.e. glycoside flavonoids, tannins, and several alkaloids. Other solvents with low polarity index dissolved lipophilic compounds such as alcana, wax, color pigments, sterols, several terpenoids and alkaloids (Seidel 2012).

Extracts of amaranth, coco-grass, and spiny amaranth contained alkaloids, tannins, flavonoids, terpenoids, and saponins as observed in Tables 4 and 5 were not surprising.

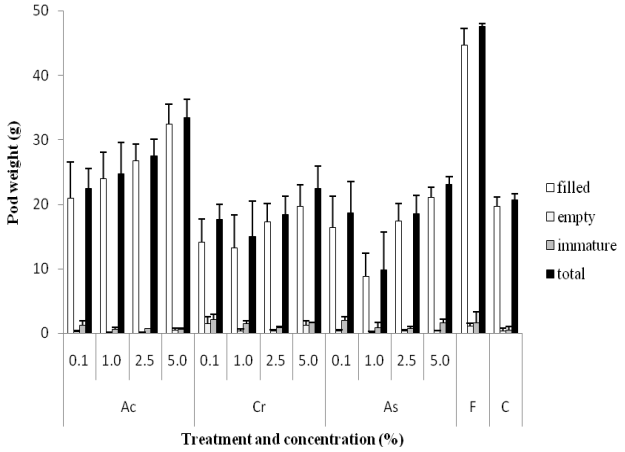

Figure 1. Distribution of filled, empty, immature, and total pod weights of peanut treated with crude extracts of *A. conyzoides* (Ac), *C. rotundus* (Cr), *A. spinosus* (As), difenoconazole fungicide (F), and water (C) based on wet basis. Concentrations the crude extracts sprayed were 0.1, 1.0, 2.5, and 5.0%. Bars represent standard errors

Table 4. Phytochemical screening tests of *A. conyzoides*, *A. spinosus* and *C. rotundus* extracts

Compound	Weed extract		
	A. conyzoides	***A. spinosus***	***C. rotundus***
Alkaloid			
a. Mayer reagent	+	-	+
b. Wagner reagent	+	+	+
Tanin			
a. $FeCl_3$	+	-	+
b. Gelatin	+	+	+
Flavonoid	++	++	++
Terpenoid	++	+	++
Saponin	+	+	++

Note: ++ more compound/darker color, + less compound/lighter color, - no compound detected.

Table 5. Number of spots of *A. conyzoides*, *A. spinosus* and *C. rotundus* extracts after separation with thin layer chromatography

Compound	Number of spots		
	A. conyzoides	***A. spinosus***	***C. rotundus***
Alkaloid	6	8	2
Tannin	1	1	1
Flavonoid	10	4	4
Terpenoid	6	8	2
Saponin	5	2	7

One of the main functions of the secondary metabolites was to protect plants from pathogen infections (Vickery and Vickery 1981; Boue et al. 2009; Mandal et al. 2010). These secondary metabolites may enhance the inhibition of the uredospore germination *in vitro* as well as to reduce the disease intensity on the infected peanut crops in the greenhouse experiment. Previous studies reported that ageratum extract inhibited the growth of *C. albicans* and *Sclerotium rolfsii* (Okunade 2002), whereas coco-grass extract was effective to control *Alternaria, Colletotrichum, Curvularia* and *Helminthosporium* (Singh et al. 2011). *Amaranthus* spp. extracts inhibited *Bacillus* spp. and *S. aureus* (Maiyo et al. 2010).

Flavonoids found in ageratum had the highest number of the spots (Table 5). This secondary metabolite may be one of the compounds which were responsible for the reduction of spore germinations or lysed spores. It had been reported that this weed contained at least 21 polygenated flavonoids which were potential to suppress the growth of pathogens (Kamboj and Saluja 2008). In their report, twenty bacteria and four fungi were inhibited by the secondary metabolites. Total inhibition occurred in *C. albicans*, *C. neoformans*, *S. rolfsii* and *Trichophyton mentagrophytes* (Okunade 2002). The concentration of 5% ageratum extract used in this study to inhibit the spore germination was less than that in Kishore and Pande's study (2005). Of the 38 plant extracts evaluated, two aqueous leaf extracts of *Prosopis juliflora* and *Lycopersicon esculentum* at a concentration of 20% (w v^{-1}) completely inhibited *in vitro* germination of both peanut rust disease and late leaf spot caused by *Phaeoisariopsis personata* (Kishore and Pande 2005).

Alkaloids inhibited growth of plant pathogens had been observed by Zhou et al. (2003). Alkaloids extracted from roots of *Veratrum taliense* (Liliaceae) inhibited growth of *Phytophthora capsici* and *Rhizoctonia cerealis* (Zhou et al. 2003). Flavonoids acted as molecule signals and as compounds for self-defense against pathogen infections and anti microbe (Boue et al. 2009; Ghasemzadeh and Ghasemzadeh 2011; Samanta et al. 2011). Saponins contain groups of glycosides with triterpene or steroid backbones. These chemicals form complex compounds with sterols, as a result, pore formation and loss of membrane integrity in plant fungal pathogens occurred (Gonzalez-Lamothe et al. 2009; Osbourn et al. 2011).

Terpenoids function as phytoalexins, i.e. antimicrobial compounds synthesized by plants in higher amount as a result of pathogen infection. Tannins may disturb protein metabolism to form hydrogen bonds, hydrophobic interactions, as well as covalent bonds (Harborne 1998; Cowan 1999; Das et al. 2010). As a result, the pathogens were not able to grow normally. The presence of alkaloids, flavonoids, tannins, saponins, and terpenoids in the weed extracts may enhance the effectiveness to control *P. arachidis*.

Ageratum extract suppressed the germination and the growth of rust disease both in laboratory as well as in greenhouse experiments. However, for a large scale of application, the use of methanol to extract the secondary metabolites may be a bit costly for some extents.

Nevertheless, the less impact on environment of the ageratum extract application may be another reason should be taken into account. Number and time of applications need to be further studied to determine the effectiveness of the extract to suppress the disease in the field.

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