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Research Paper

Screening of botanical extracts for the control of lemongrass (*Cymbopogon citratus* (DC) Stapf) rust (*Puccinia nakanishikii* L.) in green house and field condition

Mihiret Mekonnen[1]* • Beemnet Mengesha[1] • Begashaw Manhile[2]

[1]Wondo Genet Agricultural Research Center, EIAR, P.O.Box. 198, Shashemene, Ethiopia
[2]Department of Forestry College of Forestry and Natural Resource, Hawassa University, P.O. Box 128, Shashemene, Ethiopia.

*Corresponding author. E-mail: hanamihiret0@gmial.com. Fax: +251-461191245; Tel: +251-923365183 /251-911531513 251-461190702

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Abstract. *Cymbopogon citratus* is severely infected by a rust disease caused by *Puccinia nakanishikii* L. which is often responsible for drying of the leaves. The present study was undertaken to investigate the potential efficacy of various botanicals for the control of the disease. Crude acetone extract of *Lantana camara* L., *Milletia ferruginea* L., *Eucalyptus globules* L., *Maesa lanceolata* L*, Ruta chalapensis* L.*, Vernonia amygdalina* L.*, Datura stramonium* L., and *Artemisia annua* A. were investigated in greenhouse and field conditions. Plants sprayed with Impuls EC were used as standard check and untreated plants as control for both *in vivo* and field experiments. The experiment was arranged in completely randomized design and randomized complete block design for in vivo and field experiments respectively. Spore suspension of the pathogen containing 10^{-6} spores/ml was sprayed on two-month-old lemon grass seedlings. The filtered extract of each plant was sprayed separately on infected plants in the greenhouse as different treatment. For field experiment, botanicals were sprayed on well established plants before disease occurrence and continued at 15 day interval for four rounds. Data was recorded on fresh leaf yield (kg/ha), dry leaf yield (kg/ha), fresh stem wt (kg/plot), essential oil content, essential oil yield, disease severity and disease control. *D. stramonium*, *V. amygdalina* and *A. annua* were effective botanicals against *P. nakanishikii* both in the green house field experiments.

Keywords: *Cymbopogon citrates*, botanical extracts, rust disease control, essential oil yield.

INTRODUCTION

Lemongrass (*Cymbopogon citratus* (DC) Stapf) is a perennial aromatic tropical C4 grass that belongs to the family Poaceae. The genus *Cymbopogon* comprises about 140 species (Kumari et al., 2007), of which lemongrass is one of the three aromatic grasses considered economically important for the production of essential oils and aromatic herbs (Eltahir and Abuereish, 2010). It is native to South East Asian countries and it is widely cultivated in Thailand, Vietnam, Cambodia, India, Indonesian islands (Chagonda and Makanda, 2000), Africa, South America, Australia, Europe and North America (Joy et al., 2006).

It grows best under sunny, warm and humid conditions; however, higher altitudes aggravate infestation of rusts on its economically important parts i.e. all over its expanding leaves (Wijesekera, 1981). A rainfall of 2500 to 3000 mm evenly distributed throughout the year is preferred for good growth and yield of leaves (Virmani et al., 1977; Husain et al., 1988). It grows in wider range of soils, but best yields obtained from well drained sandy to

loam soils with a pH value ranging from 4.3 to 8.4 (Joy et al., 2001). However, calcareous and water logged conditions are unsuitable for its cultivation (Joy et al., 2006).

Lemongrass can be used as carminative (Kumari et al., 2009), insect repellant (Joy et al., 2006) and widely used as herbal tea (Beemnet et al., 2010). It is used to treat bronchitis, sinusitis, cold, fever, malaria, haemorrhoids, toothache, diarrhea, stomach-ache, headaches, muscular pain, digestive disorders, menstrual disorder, rheumatism and other joint pains (Simon et al., 1984). Due to its lovely fragrance, it is used as a flavouring ingredient in several products such as baby oil, massage oil, ointment for body, oil for rheumatism, oil for beauty mask, herbal baths, soap and candle making industries and herbal cooking's (Hirt and Mpia, 2001).

Lemongrass contains 1 to 2% EO on a dry weight basis, with wide variation of the chemical composition as a function of genetic diversity, habitat and agronomic treatment of the culture (Carlson et al., 2001). Its oil exhibits a broad spectrum of fungi toxicity by inhibiting several fungal species at different concentrations and its fungi toxic potency remains unaltered for 210 days of storage, after which it starts to decline. Moreover, the EO of lemongrass was superior to synthetic fungicides such as Agrosan GN, Dithane M-43 and copper oxychloride (Adegoke and Odesola, 1996; Mishra and Dubey, 1994).

Several pathogens cause diseases like leaf spot and leaf blight (*Cymbopogon* spp.) on aromatic grasses in Ethiopia (Stewart and Dagnachew, 1967; Tesfaye, 2005). Rust disease caused by *P. nakanishikii* was reported to be a serious disease on lemon grass. Heavily infected leaf tissues become discolored and necrotic in streaked patterns that correspond to the leaf veins. The rust occurs in uredinial and telial forms, the former producing lighter brown pustules than the latter. Pustules are produced on both upper and lower leaf surfaces. High rainfall, high humidity, and warm temperatures are conditions favoring disease development (Tesfaye, 2005). Wind disseminates spores among lemongrass plants (Gardner, 1985). The principal negative effects of lemongrass rust on the plant are defoliation and poor leaf development. This valuable oil bearing plant species losses its herb yield and oil quality due to the rust disease. Under dry-land conditions, the crop will produce 1 to 2 cuts a year in the rainy season. Without water it will, through its deep rooting system, survive until the next rainy season.

Environmentally friendly plant extract agents have shown to be great potential as an alternative to synthetic fungicides (Janisiewicz and Korsten, 2002; Zhang et al., 2005). Recently, the antimicrobial activity of some higher plant products that are biodegradable and safe to human health (Kumar *et* al., 2008) inviting the attention of microbiologists in the control of plant disease. Antifungal compounds from plants origin are most suitable being less toxic and more environmentally compatible by nature. A number of plants have been extracted and screened for their antifungal activities and valuable results have been achieved (El-Shami et al., 1986; Bansal and Gupta, 2000). This study was therefore focused on screening and selecting effective botanicals for the control of lemon grass rust.

MATERIALS AND METHODS

Greenhouse experiment

The greenhouse experiment was conducted at Wondo Genet College of Forestry and Natural Resource (WGCFNR), Ethiopia. Crude extracts of *L. camara*, *M. ferruginea*, *E. globules*, *M. anceolata, R. chalepensis, V. amygdalina, D. stramonium* and *A. annua* were investigated in greenhouse conditions. Plants spayed with the fungicide Impuls EC (at recommended rate) was used as a standard check and untreated plants as control. The treatments were arranged in a completely randomized design with three replication. Leaves of each plant were obtained from Wondo Genet Agricultural Research Center. They were sun dried for 3 days. Crude extract of each plant was carried out using acetone as solvent. The extraction technique used was a modification of Ruch and Worf (2001) method. One hundred and fifty gram of the dried material of each plant was soaked in 500 ml of acetone with constant stirring for 30 min and then maintained at room temperature for 24 h before being filtered. The soaked plant material was filtered with the help of a very fine and clean piece of cheesecloth separately for each plant. The filtrates were preserved in glass bottles in a refrigerator at 4°C for further use. A mixture of sterilized sandy clay loam soil**,** decomposed animal dung and sand (2:1:1 ratio) was autoclaved at 121°C for 2 h and filled into plastic pots (20 cm ×15 cm). Two slips per pot were transplanted, regularly watered and maintained in greenhouse at 26 ± 2°C and 50 to 60% relative humidity. Then, spore suspension of the pathogen containing 10^{-6} spores/ml was sprayed on two-month-old lemon grass seedlings. Then, the botanicals each at 20% concentration were sprayed on infested plants after 72 h of pathogen inoculation and continued for two months at 15 day interval.

Percent disease control (PDC) was calculated using the formula given by Wellker (1988). Estimation of disease severity was made by visual disease assessment key. Disease intensity was assessed using 0 – 4 points rating scale, where 0 = no disease (healthy); 1 = 1 to 25%, 2 = 26 to 50%; 3 = 51 to 75%; 4 = 76 to 100% leaf areas infected, using the following formula:

$$PDC = \frac{DC - DT}{DC} \times 100$$

Where, PDC: percentage disease control, DC: disease in

Table 1. Efficacy of botanical extract for the control of rust on lemongrass in green house condition.

No.	Treatments	Disease severity%	Disease control %
1	*L. camara*	20.33[b]	35.48[h]
2	*D. stramonim*	10.00[dc]	67.70[b]
3	*E. globules*	16.00[c]	48.38[f]
4	*V. amygdallina*	11.66[d]	62.38[c]
5	*M. lanceolata*	15.00[c]	51.60[e]
6	*R. chalepensis*	19.66[c]	38.70[g]
7	*A. annua*	11.00[d]	64.50[c]
8	*M. ferruginea*	13.33[d]	57.89[d]
9	Impulse	5.00[f]	83.00[a]
10	Control	31.00[a]	0.00[i]
	Cv	7.8	8.4
	LSD	2.2	3.34

Means with the same letter within the same column are not statistically different ($p < 0.05$).

control, DT: disease in treated plants.

Field experiment

The experiment was conducted at Wondo Genet Agricultural Research Center experimental field, Ethiopia. The site is located at 7° 192′ N latitude and 38° 382′ E longitudes with an altitude of 1780 m above mean sea level. The site receives a mean annual rainfall of 1000 mm with minimum and maximum temperatures of 10 and 30°C, respectively. The soil textural class is clay loam with an average pH of 7.2 (Tesfaye, 2005). The experiment was laid out in randomized complete block design (RCBD) with three replications. A plot size of 3 m × 3 m with 60 cm × 60 cm spacing between plants was used. Spacing between plots and blocks were 1m and 1.5 m, respectively. Only those effective plant extracts during greenhouse experiment were further investigated under field condition. The control and the standard chemical used were same as in the greenhouse experiment.

After well establishment of the plants, crude extracts (20% concentration) of each plant was sprayed before disease occurrence and continued for three months at 15 day interval. Data was recorded on fresh leaf yield (kg/ha), dry leaf yield (kg/plot), fresh leaf essential oil content, essential oil yield (%) and disease severity (a visual estimate of percent leaf area per plant covered by lesions). Percent disease control (PDC) was calculated using the formula given by Wellker (1988).

$$PDC = \frac{DC - DT}{DC} \times 100$$

Where, PDC: percentage disease control; DC: Disease in control; DT: Disease in treated.

Essential oil content was determined on a dry weight basis from 250 g of composite leaves harvested from three middle rows of a plot. Five samples were taken from each plot. Laboratory analyses were performed at Wondo Genet Agricultural Research Center. Essential oil was determined by hydrodistillation (Guenther, 1972). Experimental data was statistically analyzed by analysis of variance (ANOVA) using SAS PROC GLM (2002). Difference between means was assessed using Duncan's multiple range tests at $P < 0.05$.

RESULT AND DISCUSSION

Table 1 shows the effect of foliar application of plant extracts on the control of lemongrass leaf rust in greenhouse condition. The results of the green house experiment showed that extracts of *D. stramonium*, *V. amygdallina* and *A. annua* had significant potential in controlling lemongrass rust. In contrast, *L. camara* and *R. chalepensis* were not effective in controlling lemongrass rust. Consequently, high disease severity was recorded on plants sprayed with extracts of these plants. However, the rust disease was significantly reduced by applying extracts of these plants compared to the control. Acetone plant extracts showed better inhibitory activity than other solvents. This is because most of the organic soluble chemicals in plants can be extracted in the acetone. On the other hand, water fails to extract non-polar active compounds in plant materials (Masoko et al., 2005). These authors also reported that acetone extracts were superior to other extraction solvents such as hexane, dichloromethane and methanol.

Table 2. Efficacy of different botanicals for the control of lemongrass leaf rust (*P. nakanishikii*).

No.	Treatment	FLWPP	FLWHA	DLWPP	EOC	EOY	DSE	DC
1	*M. lanceolata*	17.40^{c}	1656^{c}	16.35^{c}	0.544^{a}	9.465^{d}	16.83^{c}	42.93^{d}
2	*M. ferruginea*	15.20^{d}	1368^{d}	13.511^{d}	0.632^{a}	9.606^{d}	19.75^{c}	33.051^{e}
3	*A. annua*	20.60^{c}	1674^{c}	16.53^{c}	0.561^{a}	11.556^{c}	13.16^{d}	55.36^{c}
4	*D. stramonium*	24.06^{b}	1986^{b}	19.61^{b}	0.573^{a}	13.786^{b}	12.00^{d}	59.32^{b}
5	*E. globules*	16.00^{d}	1260^{d}	12.44^{d}	0.508^{a}	8.128^{d}	16.75^{b}	43.22^{d}
6	*V. amygdallina*	21.46^{bc}	1842^{bc}	18.19^{bc}	0.506^{a}	10.858^{c}	12.25^{e}	58.47^{b}
7	Impulse	26.93^{a}	2424^{a}	23.94^{a}	0.564^{a}	15.231^{a}	1.03^{f}	96.49^{a}
8	Control	13.00^{d}	1170^{d}	11.55	0.601^{a}	7.047^{e}	29.50^{a}	0.00^{f}
	CV	7.4	7.4	7.4	21.34	22.42	3.326	3.53
	LSD	2.409	216.83	2.141	0.209	4.140	0.8746	2.9647

Means with the same letter within the same column are not statistically different *($p < 0.05$)*. Where, FLWPP – fresh leaf weight per plot, FLWHA – fresh leaf weight per hectare, DLWPP- Dry leaf weight per plot, EOC - essential oil content, EOY - essential oil yield, DSE- disease severity and DC disease control.

Our findings corroborate with the earlier findings of many researchers. Hussain et al. (1992) reported that the leaf extract of *D. stramonium* reduced the development of rust pustules on the leaves of wheat. The inhibitory effect of the plant extract might be attributed to the presence of antifungal compounds. The results obtained with *D. stramonim* was also in conformity with the report of Nidhi and Trivedi (2002) who have reported that the leaf extract of *L. inermis* was effective against mycelial growth of cumin wilt caused by *Fusarium oxysporum* f. sp. Similarly, antifungal activity of *V. amygdallina* leaf extract was reported by Wedge et al. (2000) and Alabi et al. (2005). Fresh leaf extract of *M. lanceolata* was identified as an effective botanical plant for the control of covered smut of sorghum. Its performance was similar to the standard fungicide Tiram (Aschalew et al., 2012).

In field condition, application of crude extracts of *D. stramonium, V. amygdallina* and *A. annua* showed virtually similar efficacy with the results obtained in greenhouse condition against *P. nakanishikii*. Significant differences ($P < 0.05$) were observed between the treated and control groups for most of the measured parameters including fresh leaf yield, dry leaf yield, fresh stem weight and essential oil yield (Table 2).

Generally, fresh leaf weight and essential oil yield were higher in treated plants than untreated control. The disease severity levels were high on untreated plants with corresponding decrease in essential oil yield. There was a clear negative correlation between the level of disease severity and essential oil yield (Table 2). Relationships of essential oil and metabolic constituents of lemongrass are linked with sugars, peroxidase enzyme and protein (Ghosh and Chatterjee, 1976). According to these authors' report, lower protein content was recorded during maximum essential oil formation in lemongrass. The results of this study indicated that as the rust infestation increased, the values of all measured variables were decreased. Among the tested plant extracts, *D. stramonium* was found to be most effective against lemongrass rust when applied at 20% concentration and reduced disease severity by 21% compared to the control. This was followed by *A. annua* and *V. amygdallina* which reduced disease severity by 20%. But the efficacy of *D. stramonium* was low as compared with the synthetic fungicide Impulse EC.

The better efficacy of *D. stramonium* acetone extract over the other plant extracts may be attributed to its quality antifungal active compounds. Tabil (1995) reported that the qualitative and quantitative differences of biologically active compounds among plant species account for their effectiveness. Anti-fungal activity of different plant extracts has been reported earlier by several investigators against a number of plant pathogens (Hassan et al., 2005; Yang and Clausen, 2007). Amadioha and Obi (1999) demonstrated the antifungal activity of seed extract of Neem and *Xylopia aethiopica* against *Colletotrichum lindemuthianum* on cowpea. Saha (1997) also reported that leaf extract of *Lawsonia inermis* completely controlled the growth of *Drechslera oxyzae*, *Sclerotium oryzae*, *Sclerotium rolfsii* and *Rhizoctonia solani* at 20% (w/v) concentration. Generally, the results of the present study demonstrated that, foliar application of leaf extracts of *D. stramonium*, *V. amygdalina* and *A. annua* significantly reduced the rust disease on lemongrass.

CONCLUSION

In this study, fresh leaf extracts of *D. stramonium, V. amygdalina* and *A. annua* were identified as effective botanical extracts for the control of lemongrass leaf rust (*P. nakanishikii*). The use of those botanicals is cost effective and environmentally safe and is potentially useful for resource poor small-scale farmers as it is locally available. However, further study should be conducted at higher and various concentrations to determine their maximum possible efficacy for the control

of lemongrass rust.

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