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Screening of Botanical Extracts for the Control of Spearmint Leaf Rust in Greenhouse and Field Conditions

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Abstract: Spearmint (*Mentha spicata* L.) is a short-term perennial herb in Lamiaceae family. Mint production is affected by many diseases among which being highly susceptible to leaf rust (*Puccinia menthae*) that causes up to 70% yield loss in the study area. The present study was undertaken to investigate the potential efficacy of various botanicals for the control of spearmint leaf rust. 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 *C. citrates* (DC) Stapf were investigated in greenhouse and field conditions. Plants sprayed with propiconazole were used as standard check and untreated plants as control. The experiment was arranged in completely randomized design (CRD) and randomized complete block design (RCBD) both for in vivo and field experiments, respectively. Spore suspension of the pathogen containing 10^{-6} spores/ml was sprayed on two-month-old spearmint seedlings. Filtered extract of each plant was sprayed separately on infected plants in greenhouse as different treatment. For field experiment, botanicals were sprayed on well established plants before disease occurrence and continued for two months at 15 day interval. Data was recorded on Fresh leaf yield (kg ha^{-1}), dry leaf yield (kg ha^{-1}), fresh stem wt (kg/plot), essential oil contents, essential oil yield, disease severity and disease control. *D. stramonium, M. lanceolata* and *M. ferruginea* were found significantly effective botanical extracts both in green house and field condition for the control of spearmint leaf rust.

Key words: *Mentha spicata* · Botanical Extracts · Rust Disease Control · Essential Oil Yield

INTRODUCTION

Spearmint (*Mentha spicata* L.) is a short-term perennial herb in Lamiaceae family. Lamiaceae is a family of flowering plants that comprises over 240 genera and 6,500 species worldwide. Spearmint belonging to the genus Mentha, is native to the Balkan Peninsula and Turkey and it has been naturalized throughout much of Europe, the Mediterranean region, Southwest Asia and other parts of the world [1, 2]. It grows as a short-term perennial herb, the first year as a row crop and the next three to four years as a field crop. It is shallow-rooted and requires loose-textured soils for good root penetration and growth. It requires abundant moisture and relatively high fertility (especially nitrogen) for optimum growth [3]. It is also cultivated in gardens throughout much of the world as food herbs and medicinal herbs [2, 4 and 5]. Its height ranges from 33 to 58 cm and the plant is said to grow well in wet semi shade conditions. Its leaves are in the form of slender spikes, oblong or lanced in shape, measuring over two inches in the length and of bright greenish color.

Spearmint has been cultivated for thousands of years for the unique fragrances produced by its volatile oils. The genus *Mentha* contains more than 45 accepted species and sub-species. However, commercial production of mint oil is based primarily on *M. arvensis* (Corn Mint), *M. spicata* (spearmint) and *M. piperita* (peppermint). The world market for spearmint oils is approximately 1500 tons/yr and increasing at approximately 5% per year.

Spearmint was originally used as a medicinal herb to treat stomach ache, common cold and chest pains. Its tea is a strong diuretic. The leaves are often used by many campers to repel mosquitoes and insect pests. The essential oil is used as an environmentally-friendly insecticide for its ability to kill some common pests like mosquitoes, wasps, hornets, ants and cockroaches.

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Menthol from spearmint essential oil (40-90%) is an ingredient of many cosmetics and some perfumes. Menthol and the essential oil are also much used in medicine as a component of many drugs and are very popular in aromatherapy [6]. The oil obtained from the leaves is widely used to flavor gum, candy and various pharmaceutical preparations [7]. Some dishes commonly using spearmint are lamb, fish, mint tea, candy, tabouli salad and the ever popular mint jelly.

In spite of the importance of spearmint in many areas around the world and its use in medicine, food and landscaping, spearmint production is basically affected by many diseases. Worldwide known and important diseases are powdery mildew (*Erysiphe cichoracearum*), leaf spot (*Curvularia lunata*), leaf blight (*Alternaria spp*.), wilt (*Verticillium albo-otrum*), stolen rot (*Rhizoctonia bataticola*), collar rot (*Sclerotium rolfsii*), bacterial leaf spot (*Pseudomonas cichorii*), antracnose (*colletotrichum gloeospcrioides*) and rust (*Puccinia menthae*) [8]. Among all diseases, leaf rust (*Puccinia menthae*) has been found the most abundant diseases of spearmint crop at Wondo Genet experimental field [9]. Spearmint rust has caused major problems for the spearmint growers. If uncontrolled, the disease reduces oil yield by 50% or more and also reduces oil quality. Although rust affects some shoots in the early spring, it usually is noticed first on older plants as orange to reddish-brown spots on the underside of the lower leaves. In late summer and fall, the rust disease appears as dark brown spots on the leaves of plants, as the fungus produces spores that over winter. Rusted leaves turn brown and drop off the plants. Defoliation can be severe late in the season, with accompanying loss in oil yields. Moderate to severe rust infestations weaken the plants and reducing winter survival [10]. When the brown spore masses break through the leaf surface, oil-bearing glands on that portion of the leaf are destroyed. Since, 99% of the oil glands are on the leaves, the formation of the brown spore causes serious losses of oil. Infected spearmint stands can suffer a 70% yield loss [8]. This pathogen can spread rapidly to healthy plants and can be carried by wind for long distances. Since spearmint rust survives as teliospores on mint foliage, it can be introduced in a new stand when rootstock is dug from infested stands [6].

The application of protective chemical fungicides prior to disease occurrence has been one of the common practices against the disease outbreak. However, problems associated with the use of hazardous chemicals for plant disease control causes health hazards, environmental pollution, pathogens' resistant to chemical pesticides and ecological imbalances [11]. Botanical extracts have been reported to be effective against diseases of many crops [12]. These are natural products that can even be used in their crude forms. They are far low toxic to non-target organisms, biodegradable and environmentally safe [13].

Research on botanical control of plant pathogens has received much attention in recent years as a means of increasing crop production by avoiding a number of problems related to chemical control. Among different strategies for plant growth promotion and disease suppression, botanical control approaches are very useful and an ominous choice in managing plant diseases [14]. Therefore, the present study was mainly undertaken to explore the potential efficacy of various botanicals for the control of spearmint leaf rust as an alternative to chemical control.

MATERIALS AND METHODS

Greenhouse Experiment: The greenhouse experiment was conducted at Wondo Genet College of Forestry and Natural Resource (WGCFNR), Ethiopia. Crude acetone extract of *L. camara*, *M. ferruginea*, *E. globules*, *M. anceolata, R. chalepensis, V. amygdalina, D. stramonium* and *C. citrates* were investigated in greenhouse conditions. Plants sprayed with propiconazole (Tilt) were used as a standard check and untreated plants as control. The treatments were arranged in a completely randomized design (CRD) with three replications each. Leaves of each plant were obtained from Wondo Genet Agricultural Research Center. They were sun dried for 2 days. Crude extract of each plant was carried out using acetone as solvent. The extraction technique used was a modification of [15] method. One hundred gram of the dried material of each plant was soaked in 500 ml of acetone with constant stirring for 30 minutes and then maintained at room temperature for 24 hours 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 2hr and filled into plastic pots (20 ×15cm). Two stolon segments (10-12 cm) were transplanted, regularly watered and maintained in greenhouse at 26 ± 2 °C and 50-60% relative humidity. Then, spore suspension of the pathogen containing 10^{-6} spores/ml was sprayed on two-month-old seedlings.

Then, the botanicals each at 40% concentration were sprayed on infested plants after 72 hours of pathogen inoculation and continued at 15 day interval for four rounds.

Disease severity was assessed using a visual assessment key (visual estimate of percent leaf area per plant covered by lesions) adapted for spearmint rust by [16] 6 weeks after inoculation. Percent disease control (PDC) was calculated using the formula given by [17].

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

Where,
PDC– percentage disease control;
DC– disease in control and
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 380°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 [9]. The experiment was laid out in randomized complete block design (RCBD) with three replications. A plot size of 3 x 3m with unknown spacing between plants was used as Japanese mint has creping nature. Spacing between plots and blocks were 1m and 1.5m, respectively. Only those effective plant extracts during greenhouse experiment were further investigated under field condition. Crude extracts of *M. ferruginea*, *E. globules*, *M. anceolata, V. amygdalina, D. stramonium* and *C. citrates* were investigated in field conditions. The control and the standard chemical used were same as in the greenhouse experiment. Stolon segments from adjacent experimental plots were planted in rows. After well established the plants, crude extracts (40% concentration) of each plant was sprayed 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/plant, fresh stem wt.(kg/plant), fresh leaf essential oil content and essential oil yield (%) after six months. Disease severity was assessed after 3 months of planting. Percent Disease Control (PDC) was calculated using the same mentioned above formula given by [17].

Essential oil content was determined on a dry weight basis from 250g of composite leaves harvested from three middle rows of a plot by hydro- distillation [18]. Five samples were taken from each plot. Data was statistically analyzed using analysis of variance (ANOVA) and difference between means was assessed using Duncan's Multiple Range Test at 5% probability level using SAS statistical software [19].

RESULTS AND DISCUSSION

The results of the experiment revealed that all the eight botanical extracts showed significant differences ($P > 0.05$) compared to the control (Table 1). Maximum disease control (65.70%) was recorded in *D. stramonium and M. ferruginea* sprayed plots. However, *M. lanceolata and C. citrates* extracts were also able to suppress the disease to 60 and 57.14% respectively under field condition (Table 1). The standard fungicide (Tilt) showed best (96.84%) efficacy compared to all botanical extracts. On the other hand, *R. chalepensis* and *L. camara* showed relatively lower efficacy than other leaf extracts each with 42.85% efficacy. Promising crude extracts verified during greenhouse evaluation were further evaluated in field condition.

For the field experiment, only those effective botanicals during greenhouse experiment were investigated. Among investigated botanicals, the maximum efficacy (70%) was recorded in *D. stramonium*. Foliar application of aqueous leaf extract of this plant significantly reduced the rust disease while not affecting the growth of the plant negatively. Consecutive sprays of *D. stramonium* extract (40%) at 15 days interval starting from the initiation of the disease have significantly reduced the rust disease by 70% and increased the essential oil yield by 5.62% compared to the control.

Our finding with *D. stramonium* leaf extract was in conformity with the report of [20] who reported that leaf extract of *D. stramonium* reduced the development of rust pustules on leaves of wheat. Similar result was reported by [21] on the use of *C. gigantea* and *P. juliflora* extracts against *A. helianthi*, the causal agent of alternaria blight on sunflower. Results of the present study showed that leaf extracts of *M. lanceolata*, *M. ferruginea*, *C. citrates* and *V. amygdalina* were potentially reduced the disease severity with 69.70, 66.36, 55.75 and 54.54% efficacies, respectively. The obtained results were suitably correlated with results of other workers on rust disease control [22, 23].

Like the greenhouse experiment, the standard fungicide (Tilt) showed the best (96.24%) efficacy compared to all botanical extracts (Table 2).

Table 1: Efficacy of botanical extracts for the control of spearmint leaf rust in greenhouse condition.

No.	Treatments	Disease severity%	Disease control (%)
1	*Lantana camara*	20.00^{b}	42.85^{f}
2	*Milletia ferruginea*	12.00^{de}	65.70^{b}
3	*Eucalyptus globules*	18.00^{c}	48.50^{d}
4	*Mesa lanceolata*	14.00^{d}	60.00^{c}
5	*Vernonia amygdallina*	18.00^{c}	48.50^{d}
6	*Ruta chalepensis*	20.00^{b}	42.85^{f}
7	*Datura stramonium*	12.00^{de}	65.70^{b}
8	*Cympopogon citrates*	16.00^{d}	57.14^{d}
9	Tilt	1.00^{f}	96.84^{a}
10	Untreated check	35.00^{a}	0.00^{g}
	CV	9.78	7.24
	LSD	2.32	6.54

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

Table 2: Efficacy of different botanicals for the control of *p. menthae* in field condition.

Treatments	FLWPP	FLWHA	DLWPP	FSWPP	DSWPP	EOC	EOY	DS (%)	DC (%)
Mesa lanceolata	21.07^{cd}	1516.8^{cd}	16.85^{cd}	24.86^{abcd}	19.89^{abcd}	0.537^{a}	11.28^{bc}	8.33^{d}	69.70^{b}
Milletia ferruginea	20.00^{d}	1440.0^{d}	16.00^{d}	22.93^{bcd}	18.347^{bcd}	0.523^{a}	10.46^{bc}	9.25^{d}	66.36^{c}
Cymbopogon citrates	23.06^{bc}	1060.8^{e}	11.78^{e}	20.40^{de}	16.32^{de}	0.562^{a}	8.82^{de}	12.16^{c}	55.75^{d}
Datura stramonium	25.00^{b}	1800.0^{b}	20.00^{b}	26.53^{ab}	21.22^{ab}	0.497^{a}	12.26^{b}	8.25^{d}	70.00^{b}
Eucalyptus globules	17.33^{e}	1080.0^{e}	12.00^{e}	20.66^{cde}	16.53^{cde}	0.562^{a}	9.66^{cd}	13.75^{b}	50.00^{e}
Vernonia amygdalina	23.06^{bc}	1660.8^{bc}	18.45^{bc}	25.46^{abc}	20.37^{abc}	0.482^{a}	11.14^{cd}	12.5^{c}	54.54^{d}
Tilt	35.06^{a}	2524.8^{b}	28.05^{a}	29.13^{a}	23.30^{a}	0.444^{a}	15.58^{a}	1.03^{e}	96.24^{a}
untreated check	12.86^{f}	868.8^{f}	9.65^{f}	15.93e	12.74^{e}	0.518^{a}	6.64^{e}	27.50^{a}	0.00^{f}
CV	7.09	7.08	7.08	12.38	12.38	16.67	12.939	12.84	10.385
LSD	2.64	185.26	2.058	5.04	4.03	0.15	2.43	2.765	10.055

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

Where, FLWPP- fresh leaf weight per plant, FLWPA- fresh leaf weight per hectare, DLWPP- dry leaf weight per plant, FSWPP- fresh stem weight per plant, DSWPP- dry stem weight per plot, EOC- fresh base essential oil content, EOY- essential oil yield (%) DS- Disease severity and DC- disease control (%).

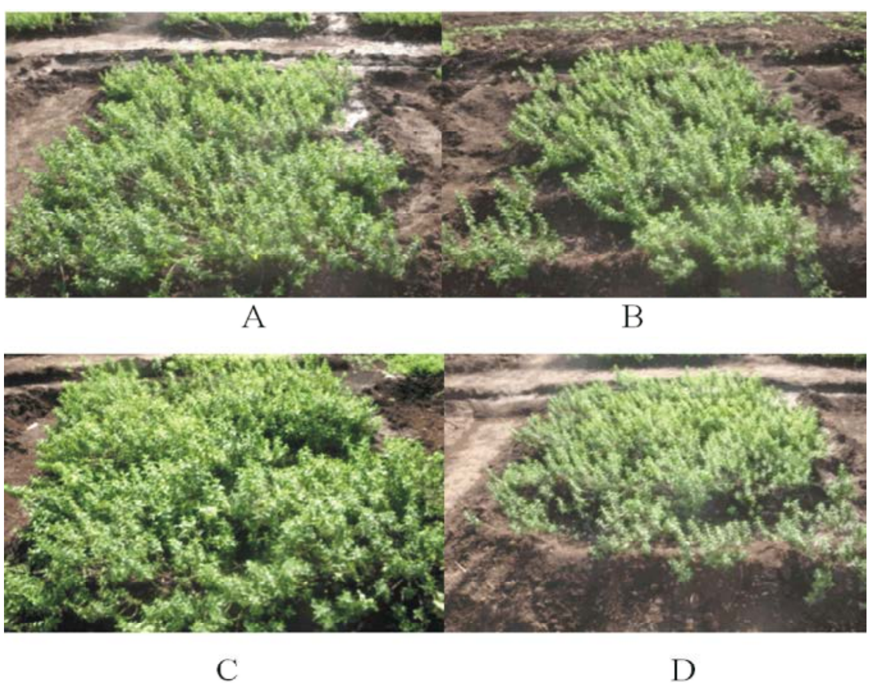

Fig. 1: Effect of botanical extracts on the control of spearmint leaf rust: (A) *M. lanceolata* (B) *M. ferruginea* (C) *D. stramonium* (D) Untreated.

However, all botanical extracts were significantly superior to the control (P<0.05) in all parameters measured (fresh leaf yield, dry leaf yield, fresh stem wt. and essential oil yield). [24] Reported that *P. juliflora* leaf extract was highly effective in inhibiting growth of rust disease on sunflower. The present study results revealed that spearmint rust significantly reduced fresh leaf weight and essential oil yield in untreated plants. During the study period, many older shoots on the rusted plants wilted irreversibly and died. Generally, fresh leaf weight and essential oil yield were higher in treated plants than untreated ones. The disease severity levels were higher on untreated plants with decreasing oil yield. There was a clear negative correlation between the level of disease severity and essential oil yield. Thus the above results encouraged the use of botanical extracts for the control of rust diseases on spearmint. The use of botanical extracts like lemongrass, citronella, clove, thyme and oregano oils for the control of plant disease has been employed by [25] as alternative control measures to the conventional synthetic fungicides. Similarly, fresh leaf extract of *M. lanceolata* was identified as an effective botanical extract for the control of covered smut of sorghum. Its performance was similar to the standard fungicide thiram [26].

CONCLUSIONS

The results of this study revealed that application of crude extracts of *D. stramonium*, *M. lanceolata* and *M. ferruginea* were effective against spearmint leaf rust and could be exploited as an alternative to synthetic fungicides. However, *D. stramonium* was most effective botanical against spearmint rust (*P. menthae*) next to the standard chemical (Impulse EC) both in greenhouse and field conditions. There is a need for further research with these crude extracts at varying concentrations to determine the least concentration effective against the target disease to assist possible commercial formulations with minimal waste.

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REFRENCES

1. Kokkini, S. and D. Vokou, 198. *Mentha spicata* (Lamiaceae) Chemotypes Growing Wild in Greece. Eco. Botany, 43(2): 192-202.
2. eFloras, 2008. Published on the Internet http:// www.efloras.org [accessed 6 December 2010]. Missouri Botanical Garden, St. Louis, MO and Harvard University Herbaria, Cambridge, MA.
3. DiTomaso, J. and E. Healy, 2007. Weeds of California and Other Western States. Vol. 2. University of California Agriculture and Natural Resources Communication Services, Oakland, CA., pp: 974.
4. Abbaszadeh, B., H. Farahani, S. Valadabadi and P. Moaveni, 2009. Investigations of variations of the morphological values and flowering shoot yield in different mint species at Iran. J. Hort. and Fore. 1(7): 109-112.
5. Lawrence, B., 2006. Mint: The genus *Mentha*. CRC Press, Taylor and Francis Group. Boca Raton, FL., pp: 576.
6. Baird, C.R., H. Homan and E. Eric, 1993. Insect control recommendations for mint_production in Idaho. Coop. Ext. Bull. 773. Coll. Of Ag. University of Idaho, Moscow Idaho.
7. Beemmnet M., M. Omarsherif, T. Tsion and A. Solomon, 2009. Production, processing and utilization of Aromatic plant. (EIAR), Ethiopian Institute of Agriculture Research (EIAR), Addis Ababa, Ethiopia, pp: 31.
8. Bienvenu, F.E., 1992. Development of a viable peppermint oil industry in south eastern Australia. Rural Industries Research and Development Corporation Report DAV 24A.
9. Tesfaye, B., 2005. Survey report on pests of essential oil in Ethiopia (unpublished).
10. Lacey, M.L., T.S. Christine, J.G. Ralph and Y. Alan, 2000. Mint Production in the Midwestern United States.
11. Fry, W.E., 1982. Principles of plant disease management. Academic Press, New York, pp: 378.
12. Wang, W.Q., B.H. Daniel and Y. Cohen, 2004. Control of plant diseases by extracts of Inula viscosa. J. Phytopathol, 94: 1042-1047.
13. Neupane, F.P., 2003. Integrated pest management in Nepal. Centre for Environmental and Agricultural Policy Research, Extension Development (CEAPRED), Lalitpur, pp: 77-96.

14. Wilson, C.L and J.M. Solar, 1997. Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Disease, 81: 204-210.
15. Ruch, B.A. and R. Worf, 2001. Processing of neem for plant protection simple and sophisticated standardized extracts. Abstracts of the. Work shop, Neem and Pheromones, University of Uberaba, Brazil, March 29-30 Augusts, pp: 499.
16. Beresford, R.M. and Mulholland, 1987. Mint rust on cultivated peppermint in Canterbury: disease cycle and control by flaming. New Zealand. J. Exp. Agric., 15: 229-233.
17. Wellker, D.M., 1988. Biological control of soil borne plant pathogens in the rhizosphere whit bacteria. Annual Review of Phytophathol, 26: 379-407.
18. Guenther, E., 1972. The Essential oils; History origin in plants production analysis (vol1), Robert E. kriger publishing Co., Malabar, Florida, pp: 427.
19. SAS (Statistical Analysis System), 2002. SAS/ STAT. Guide version 9. SAS, Institute Inc. Raleigh, Vorth Carolina,USA.
20. Hussain, I., M.A. Nasir and M.R. Haque, 1992. Effect of different plant extract on brown rust and yield of wheat. J. Agric. Res., 30: 127-131.
21. Mesta, R.K., V.I. Benagi, K. Srikant and I. Shankergoud, 2009. In vitro evaluation of fungicides and plant extracts against Alternaria helianthi causing blight of sunflower. Karnataka J. Agric. Sci., 22(1): 111-114.
22. Sunkad, G., S. Kulkari and V.I. Benagi, 2005. Effectiveness of neem seed kernel extract in combination with selected fungicides for groundnut rust management. Int. Arach. Newsletter, 25: 39-41.
23. Usman, M.R., R. Jaganathan and D. Dinakaran, 1991. Plant disease management if groundnut with naturally occurring plant products. Madras Agr. J., 78: 152-153.
24. Anonymous, A., 2006. Annual Progress Report of Sunflower. Directorate of Oilseeds Research Hyderabad, pp: 56-64.
25. Viudamartos, M., Y. Ruiz-navajas. J. Fernandez-lopes and J.A. Perez-alvarez, 2007. Antifungal activities of thyme, clove and oregano essential oils. J. Food Saf., 27: 91-101.
26. Aschalew, S., A. Fekede and W. Kedir, 2012. Evaluation of three potential botanicals against sorghum covered smut (*Sphacelotheca sorghi*) at Bako, Western Oromia Ethiopia. Available: http://www.geog.ubc.ca/biodiversity/eflora/index.shtm.