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Full Length Research Paper

Screening of Botanical extracts for the control of Japanese mint (*Mentha arvensis* L.) Leaf rust *(Puccinia menthae)* in Greenhouse and field condition

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Mentha arvensis **is a robust perennial aromatic and medicinal herb that belongs to the Labiatae family. Its production is affected by many diseases. Among all diseases,** ***Puccinia menthae*** **has been found the most abundant diseases of Mint crop at the study site. The study was conducted to screen effective botanicals for the control of Japanese mint leaf rust. Crude extracts of** ***L. camara*****,** ***M. ferruginea*****,** ***E. globules*****,** ***M. anceolata, R. chalepensis, V. amygdallina, D. stramonium*** **and** ***C. citrates*** **were evaluated for their efficacy. Propiconazole was used as a standard check and untreated plants as control. Treatments were arranged in completely randomized design and randomized complete block design for greenhouse and field experiments, respectively. The milled material (150gm) of each plant was treated with 150 ml of acetone as extract ant. Spore suspension of the pathogen containing 10^{-6} spores/ml was sprayed on two-month-old Japanese Mint seedlings. The filtered extract of each plant at 20% concentration was sprayed. All plant extracts had shown antifungal activity against Japanese mint leaf rust. The maximum (85.56%) control was recorded in** ***M. lanceolata*****. Generally, extracts of** ***M. lanceolata*****,** ***D. stramonim*** **and** ***M. ferruginea*** **were effective to protect Japanese mint leaf rust and hence enhanced yield.**

Key word: *Mentha arvensis, Puccinia menthae***,** crude extracts, disease control.

INTRODUCTION

Mints are a group of perennial herbs belonging to the family Lamiaceae. About 20 species of the genus Mentha are cultivated in the world (Asai *et al.,* 1994). Japanese mint (*Mentha arvensis* L.) is one of the perennial herbs of this group. Owing to its high adaptation, it is most widely cultivated in the tropical and subtropical belt of the world (Everett, 1981). It is a perennial herb with shallow, creping root stocks. It is tall, downy, more vigorous variety of mint and in general has narrower leaves with a more pronounced serration. Japanese mint reproduces readily by vegetative means through underground parts called suckers. This species tolerates much drier conditions than other members of the genus that prefer a slightly acid soil and grow well in heavy clay soils. A sunny position is best for production of essential oils, but it also succeeds in partial shade (Denise, 1989).

Japanese mint is used in prescriptions for cold remedies, cough drops, dentifrices, flavoring tobacco, chewing pan, mouth washes in scenting cigarettes, bakery products and in cosmetic products (Rao, 2002; Verma *et al*., 2010).

The oil is obtained from the leaves by distillation and is widely used to flavor gum, candy and various industrial and pharmaceutical preparations (Bemmnet *et al.,* 2009). Its main constituent, menthol, is used in the manufacture of lozenges, toothpastes, pain balms, cold balms, Dabur Pudin Hara, etc. The basic raw material for mint oil production is leaves of the plant. The oil is used for treating certain stomach disorders like indigestion, gas problem, acidity, etc. The plant also is used as an insect repellent. Rats and mice intensely dislike the smell of mint. The plant was used in homes as a strewing herb and has been spread in granaries to keep the rodents off the grain.

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However, mint production is practically affected by many diseases. Worldwide known and important ones 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 leaf rust (*Puccinia menthae*) (Bienvenu, 1992). Among all diseases, leaf rust (*Puccinia menthae*) has been found the most abundant diseases of Mint crop in the study area, Wondo Genet Agricultural Research Center, Ethiopia (Tesfaye, 2005). Study results showed that sever losses in herbage yield (20-35%) has occurred due to the loss of leaves in rust infected plants and the essential oil production is drastically reduced (Ganguly and Pandotra, 1962). The infestation process is favored by overhead irrigation, which frequently allows water to stand on plant leaves long enough for the fungal spores to germinate. Closely planted mints are at a heightened risk due to increased humidity around the plants.

Rust on mint plants looks similar to other rusts in later stages, with orange to rust-colored spots covering the undersides of lower leaves in early spring. In a report by Bienvenu (1992), rust causes light-yellow, blister-like lesions appear on young shoots in the spring. Later in the season, brownish-red spots surrounded by a yellow halo appear on the leaves. In late summer and fall, the spots on the leaves become deep-chocolate brown, as the over wintering spores of the fungus are produced.

Chemical control apart from being expensive and toxic to handlers and environment, some insect pests now show resistance; therefore, the use of botanical compounds may serve as a better substitute (Gold and Messiaen, 2000). Essential oils (EOs) of some plants have recently proved to be successful bio-control agents that are nonhazardous, easily biodegradable and eco-friendly (Cox *et al.,* 2000; Chutia *et al*., 2006; Sokovic and Griensven, 2006). One of the recent approaches for plant disease management is exploitation of plant products. Among the various alternatives, natural plant products are catching the attention of scientists worldwide. Such products from higher plants and microbes are relatively broad-spectrum, bio-efficacious, economical, and environmentally safe and can be ideal candidates for use as agrochemicals (Macias *et al*., 1997; Cutler 1999). Among these, essential oils from a number of plants have been reported to show activity against a wide array of plant pathogenic fungi (Rice, 1995). These are relatively safe to the user and the environment (Wilson *et al*., 1997).

Rust epidemics can be explosive and once out of control can be difficult to manage. In Ethiopia, at Wondo Genet Agricultural Research Center experimental field, rust infestation on the leaves of Japanese mint was reached up to 30%. Most of the time, all leaves dry and fall down totally before the plant reach for harvest. Despite the disease is one of the serious production problems of Japanese mint in the area, no botanical screening activities and other control mechanisms has been devised in the past years. Hence the aim of this study was to screen suitable botanicals for the control of Japanese mint leaf rust as an alternative approach to fungicides towards improved management activities.

MATERIALS AND METHODS

Greenhouse Experiment

The greenhouse experiment was conducted at Wondo Genet College of Forestry and Natural Resource (WGCFNR), Ethiopia. The pots were maintained in greenhouse at 26 ± 2 °C and 50–60% relative humidity. Crude extracts of *Lantana camara L.*, *Milletia ferruginea* L., *Eucalyptus globules* L., *Mesal anceolata* L.*, Ruta chalepensis* L.*, Vernonia amygdalina* L.*, Datura stramonium* L. and *Cymbopogon citrates* (DC) Stapf were investigated in greenhouse conditions. Plants spayed with propiconazole (Tilt) were used as a standard check and untreated plants as control. The treatments were arranged in a completely randomized design with three replications. Leaves of each plant were obtained from Wondo Genet Agricultural Research Center. They were sun dried for 3 days. Crude extraction of each plant was carried out using acetone as extract ant. The extraction technique used was a modification of Ruch's (2001) method. One hundred Fifty gram of each of the dried material were treated with 500 ml of acetone with constant stirring for 30 minutes and then maintained at room temperature for 24 hours before being filtered. After that, the acetone and grounded materials were filtered with the help of a very fine and clean piece of cheesecloth separately for every plant species. Then, the extracts (stock) were preserved in glass bottles in a refrigerator at 4ºC for further use. Then**,** a mixture of sterilized sandy clay loam soil**,** decomposed animal dung and sand (2:1:1 ratio) was autoclaved at 121^0C for 2hr and filled into plastic pots (20cm ×15cm). Two stolon segments (10-12 cm) per pot were transplanted and were regularly watered. 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.

Estimation of how much leaf area affected by mint rust was made by comparing leaves with a visual disease assessment technique. Disease severity was calculated as the mean percentage loss of leaf area per mint stem, using a visual assessment key adapted for spearmint rust by Beresford and Mulholland (1987). Disease severity (a visual estimate of percent leaf area per plant covered by lesions) was assessed 6 weeks after inoculation. 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 and DT– disease in treated plants.

Field Experiment

The experiment was conducted at Wondo Genet Agricultural Research Center (WGARC), Ethiopia. The site receives a mean annual rainfall of 1000mm with minimum and maximum temperatures of 10 and 30^0c, respectively. The soil is clay loam with an average pH of 7.2. The experiment was laid out in randomized complete block design (RCBD) with three replications. A plot size of 3m 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. As for greenhouse experiment, crude extracts of *Milletia ferruginea* L., *Eucalyptus globules* L., *Mesal anceolata* L.*, Vernonia amygdalina* L.*, Datura stramonium* L. and *Cymbopogon citrates* (DC) Stapf were investigated in field conditions. Plants spayed with propiconazole (Tilt) were used as a standard check and untreated plants as control. Planting materials from adjacent experimental plots were lifted and stolons were broken into pieces (10-15 cm). The stolon segments were then planted in rows. After the plants well established, 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 base essential oil content and essential oil yield (%) after six months. Disease severity (a visual estimate of the percent leaf area per plant covered by lesions) was assessed after 3 months of planting. 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 and DT - Disease in treated plants.
Essential oil content was determined on a dry weight basis from 250g of composite leaves harvested from three middle rows of a plot. Five samples were taken from each plot.
Essential oil yield was determined by hydro- distillation (Guenther, 1972). 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 PROC GLM (2002).

RESULTS

Greenhouse Experiment

All plant extracts had shown antifungal activity against Japanese mint leaf rust. Among investigated botanicals, the maximum (85.56%) efficacy was recorded in *Mesa lanceolata* followed by *Datura stramonim* (73.33%) and *Milletia ferruginea* (72.20%). All other crude extracts showed comparatively lower efficacy*.* Minimum results were observed in *Lantana camara and Ruta chalepensis* with 44.4% and 41.1% efficacy respectively in greenhouse experiment (Table 1). On the other hand, the standard fungicide Tilt showed the best (96.84%) efficacy compared to all botanical extracts. However, all botanical extracts reduced the level of disease severity compared to the control. The promising botanicals during *in vivo* evaluation were further tested in field conditions.

Field Experiment

Application of the various botanical extracts showed varying potential for the control of Japanese mint leaf rust in field conditions. The results of this study indicated that extracts from semidried leaves of three botanicals, *Mesa lanceolata*, *Datura stramonium* and *Milletia ferruginea were* effective to protect Japanese mint leaf rust and hence enhanced yield. *V. amygdallina*, *D. stramonium, C. citrates, E. globules, M. ferruginea and M. lanceolata* increased essential oil yield by 8.48%, 10.83%, 11.55%, 12.59%, 13.38% and 14.68% respectively when compared to the control (3.97%). This could be because of pesticidal and inhibitory effects of the extracts. Significant difference *($P < 0.05$)* was observed between treated and untreated plants for most of the parameters measured (fresh leaf yield, dry leaf yield, fresh stem wt. and Essential oil yield) (Table 2). The rust disease significantly reduced fresh leaf weight and essential oil yield on untreated plants. During a study period, many older leaves on rusted plants wilted irreversibly and died. Generally, fresh leaf weight and essential oil yield were higher in treated plants than untreated ones (control). The disease severity levels were high on untreated plants with decreasing oil yield. There was a clear negative correlation between the level of disease severity and oil yield (Table 2).
leaf weight per plant, FLWPH- 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 (%).

DISCUSSION

Management of rust disease is unavoidable as it occurs throughout the world regardless of farming system or clim-

Table 1. Efficacy of botanical extracts for the control of on Japanese mint leaf rust in green house condition.

No.	Treatments	Disease severity%	Disease control %
1	*L. camara*	14.00^{cd}	44.40^{g}
2	*M. ferruginea*	8.30^{g}	72.20^{c}
3	*E. globules*	15.00^{c}	50.00^{f}
4	*M. lanceolata*	4.30^{h}	85.56^{b}
5	*V. amygdallina*	13.00^{e}	56.68^{e}
6	*R. chalepensis*	17.66^{b}	41.10^{h}
7	*D. stramonium*	8.00^{g}	73.33^{c}
8	*C. citrates*	10.30^{f}	65.66^{d}
9	Propiconazole (Tilt)	1.00^{i}	96.84^{a}
10	Control	30.00^{a}	0.00^{i}
	Cv	16.6	8.34
	LSD	3.67	3.89

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

Table 2. Screening of botanicals for the control of rust on Japanese mint in field condition.

Treatment	FLWPP	FLWH	DLWPP	FSWPP	DSWPP	EOC	EOY	DSE	DC
M. ferruginea L.	21.850^{b}	24278^{b}	17.48^{b}	22.28^{b}	17.82^{b}	0.611^{ab}	13.38^{bc}	8.77c	65.20^{d}
E. globules L.	25.08^{ab}	27868^{ab}	20.06^{ab}	23.07^{b}	18.45^{b}	0.502^{bc}	12.59^{bc}	13.5^{b}	46.00^{g}
M. lanceolata L.	27.55^{a}	30613^{a}	22.04^{a}	27.13^{b}	21.70^{b}	0.532^{abc}	14.68^{b}	8.00^{c}	68.00^{c}
V. amygdallina L	21.62^{b}	24030^{b}	17.30^{b}	23.40^{b}	18.72^{b}	0.400^{cd}	8.48^{de}	12.9^{b}	48.4^{f}
D. stramonium L	21.94^{b}	24380^{b}	17.55^{b}	22.48^{b}	17.98^{b}	0.493^{bc}	10.83^{c}	7.5^{cd}	70.00^{b}
C. citrates (DC) Stapf	24.99^{ab}	27773^{ab}	19.99^{ab}	24.27^{b}	19.42^{b}	0.471^{bc}	11.55^{bc}	12.0^{b}	52.00^{e}
Propiconazole (Tilt)	29.16^{a}	32410^{a}	23.33^{a}	29.06^{a}	23.24^{a}	0.671^{a}	19.55^{a}	1.00^{e}	96.00^{a}
Control	15.80^{c}	17563^{c}	12.64^{c}	15.46^{d}	12.37^{d}	0.247^{d}	3.97^{f}	25.00^{a}	0.0
CV	12.93	12.93	12.93	6.59	6.59	20.70	18.81	23.00	10.912
LSD	4.879	4.879	3.903	2.534	2.027	0.163	3.416	4.113	10.892

Means with the same letter within the same column are not statistically different ($P < 0.05$). Were, FLWPP- fresh

atic conditions. *P. menthae* causes rust disease on Japanese Mint. In the present study, the inhibitory effects of *L. camara*, *M. ferruginea*, *E. globules*, *M. anceolata, R. chalepensis, V. amygdalina, D. stramonium* and *C. citrates* were evaluated under greenhouse and field conditions. Statistically all the treatments were significantly superior to control ($P < 0.05$). The rust disease was inhibited to an extent of 70.00% in the leaf extracts of *D. stramonium*, 68.00% in *M. lanceolata*, 65.20% in *M. ferruginea* and 52.00% in *C. citrates*, 48.4 % in *V. amygdallina* and 46.00 % in *E. globules* 40 % test concentrations. However, promising botanicals should further be studied at higher and varying concentrations to confirm their maximum inhibitory potentials.

This study confirmed that mint rust substantially reduced Japanese mint growth and so the essential oil yield. Leaf loss was significantly increased, which as a consequence significantly reduced the leaf area, leaf fresh

weight and oil content of the plant. The disease likely reduced the persistence of the plant as a perennial crop at all. The findings of this work are in line with reports of previous workers. A research conducted in India indicated that beans and wheat treated with a spray prepared from tobacco containing 0.01% active compounds were almost completely protected the crops against rust disease (Elwell, 1995). Likewise, Bonaldo *et al.,* (2004) reported that the aqueous extract of *E. citriodora* was capable to induce local resistance in cucumber plants against *C. lagenarium.*

Various plant extracts including garlic, neem, *Withania somnifera* and *Acacia seyal*, mustard and horseradish have also been reported to have fungicide properties against the fungi *Penicillium digitatum (*McOnie, 1964; Samson, 1984; Obagwa, 2002; Mossini *et al*., 2009). Similarly, Saxena and Mathela (1996) reported the effectiveness of *Azadirachta indicia, Artemisia annua, Eucalyptus globules, Ocimum, Sanctum* and *Rheum emodi,* in the control of *Fusarium.* Godara and Pathak (1995) also stated that *Ocimum sanctum* leaf extract was highly effective against conidial germination of *Botryodiplodia theobromae* that causes fruit rot of sweet orange.

CONCLUSION

Among investigated botanicals, *M. lanceolata*, *D. stramonium* and *M. ferruginea* were effective candidate botanical extracts against Japanese mint rust (*P. menthae*) next to the standard chemical both in green house and field conditions. The botanical extracts showed varying antifungal activity against Japanese mint leaf rust. Further study should be conducted under typical conditions by varying the concentration of the botanicals.

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REFERENCE

Asai I, Yoshihira K, Omoto T, Sakui N, Shimomura K (1994). Growth and monoterpene production in shoot culture and regenerates of Menthe arvensis. Plant Tissue Cult*,* 11(3): 218-225.

Beemnet M, Omarsherif M, Tsion T and Solomon A (2009). Production, processing and utilization of Aromatic plant (EIAR), Ethiopian Institute of Agriculture Research (EIAR), Addis Ababa, Ethiopia. pp. 31.

Beresford RM, Mulholland RI (1987). Mint rust on cultivated peppermint in Canterbury: disease cycle and control by flaming. New Zealand Journal of Experimental Agriculture*,* 15: 229-233.

Bienvenu FE (1992). Development of a viable peppermint oil industry in south eastern Australia. Rural Industries Research and Development Corporation Report DAV 24A.

Bonaldo SM, Schwan-Estrada KRF, Stangarlin JR, Tessmann D, Scapim CA (2004). Fungitoxicity, phytoalexins elicitor activity and protection of cucumber against *Colletotrichum lagenarium*, by *Eucalyptus citriodora* aqueous extract. Fitopatologia Brasileira, 29**:**128-134.

Chutia M, Mahanta JJ, Saikia RC, Baruah AKS, Sarma TC (2006). Influence of leaf blight disease on yield of oil and its constituents of java citronella and in-vitro control of the pathogen using essential oils. World Journal of Agriculture Science*,* 2(3): 319–321.

Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, Wyllie SG (2000). The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil). J. Appl. Microbiol, 88:170–175.

Cutler HG (1999). Allelopathy in the biological control of plant diseases. *In*: Recent Advance in Allelopathy Science for the Future (Macias, F.A., Galindo, J. C. B., Molinillo, J. M. G. and Cutler, H.G. *eds*.), Vol. 1A. Servicio de Publications, Universidad de Cadiz, Spain. pp. 397-414.

Denise G (1989). The Book of Mint. Plants for a Future Database; Kangaroo Press.

Elwell H, Maas A **(**1995). Natural pest an

25014d disease control. The Natural Farming Network, Harare, Zimbabwe

Everett T (1981). The New York Botanical Garden Illustrated Encyclopedia of Horticulture, V. 7.Garland Publishing, New York, NY.

Ganguly D**,** Pandortra VR (1962). Important disease of mints and their control. Bull Regional Research Laboratory, Jammu 1(1): 52-64.

Godara SL, Pathak VN (1995). Effect of plant extracts on post harvest rotting of sweet orange fruits, Indian Journal of Mycology and plant pathology, 25(1&2), pp. 134-135.

Gold CS, Messiaen S (2000). The banana Weevil, *Cosmopohtes sordidus* Musa Pest fact Sheet, No 4, INIBAP, Montpellier, France. p. 2.

Guenther E (1972). The *Essential oils*; *History origin in plants production analysis,* Robert E. kriger publishing Co., Malabar, Florida. vol. 1, pp 427.

Macias FA, Castellano D, Oliva RM, Cross P, Torres A (1997). Potential use of allelopathic agents as natural agrochemicals. Brighton Crop Prot. Conf – Weeds*:* 33-38.

McOnie KC (1964). The latent occurrence in citrus and other hosts of a *Guignardia* easily confused with

G.citricarpa, the black spot pathogen. Phytopathology*.* 54: 40 - 43.

Mossini SAG, Carla C, Kemmelmeier C (2009). Effect of neem leaf extract and Neem oil on Penicillium growth,

sporulation, morphology and ochratoxin a production. Toxins, 1: 3-13.
Obagwa J, Korsten L (2002). Control of citrus and green and blue molds with garlic extracts. Plant Pathology, 109: 221- 225.
Rao BR (2002). Biomass yield, essential oil yield and essential oil composition of rose–Scented geranium (*Pelargonium species*) as influenced by row spacing and intercropping with corn mint (*Mentha arvensis* L.f .Piperascens malinv.ex Holmes). Industrial crops and Products, 16:133-144.
Rice EL (1995). Biological control of weeds and plant diseases. University of Oklahoma Press, Norman. pp 439
Ruch Ba, Worf R (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, p. 499.
Samson JA (1984). Tropical *fruits*- Tropical agricultural series. Longman Inc., New York, pp.64-118.
SAS (Statistical Analysis System) 2002. SAS/STAT. Guide version 9. SAS, Institute Inc.Raleigh, Vorth Carolina, USA
Saxena S, Mathela CS (1996). Antifungal activity of new compounds from *Nepeta leucophylla* and Nepetaclarkei. Applied Environ Microbiol, 702- 704.
Sokovic M, Griensven LJ (2006). Antimicrobial activity of essential oils and their components against the three major pathogens of cultivated button mushroom Agaricus bisporus. European Journal of Plant Pathology, 116: 211–224.
Tesfaye B (2005). Survey report on pests of essential oil in Ethiopia (unpublished).
Verma RS, Rahman L, Verma RK, Chauhan A, Yadav AK, Singh A (2010). Essential oil composition of menthol mint (*Mentha arvensis*) and papper mint (Mentha piperiata) cultivars at different stage of plant growth from kuma non region of west Himalaya. Open access Journal of medicinal and aromatic plant, 1: 13-18.
Wellker DM (1988). Biological control of soil borne plant pathogens in the rhizosphere whit bacteria. Annual Review of Phytophathology, 26: 379-407.
Wilson CL, Solar JM (1997). Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Disease, 81, 204-210.