Efficacy of Pepper Tree (*Schinus molle*) Extracts to Suppress Growth of *Botrytis fabae* and Manage Chocolate Spot Severity on Faba Bean (*Vicia faba*) at Sinana, Bale Zone, Southeastern Ethiopia

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Abstract: Fungal phytopathogens cause considerable crop yield reduction if not managed well. Use of synthetic chemicals is a conventional method of managing plant pathogens. However, the negative environmental impact of using synthetic chemicals prompted the search for plant-based compounds, which are relatively safer to the environment than synthetic agro-chemicals. In this study, crude extracts of pepper tree (*Schinus molle*) were evaluated through *in vitro* and *in vivo* tests to suppress growth of *Botrytis fabae* and to manage chocolate spot of faba bean (*Vicia faba* L.), respectively. Growth inhibitory effects were evaluated by applying different concentrations of aqueous, methanol and ethanol leaf extracts of *S. molle* or extraction solvents (control) using agar diffusion method in the laboratory. The laboratory experiment was done in a completely randomized design with three replications. The efficacies of leaf extracts of *S. molle* were evaluated through *in vivo* test after spraying the extracts and scoring the disease incidence and severity on *V. faba* (variety Shallo) grown in the field right after the detection of disease symptoms at 61 day after planting (DAP) up to 89 DAP on a weekly basis. The design for the field experiment was randomized complete block design (RCBD) with three replications. Mancozeb 80WP was used as a check or control treatment. Results of the *in vitro* test showed that all extract types had antifungal properties and significantly reduced mycelial growth in a concentration-dependent manner. Growth inhibitory effects also varied with extraction solvent. Results of the *in vivo* experiment showed that disease severity was significantly reduced by all types of *S. molle* extracts and particularly that of methanolic extract was on a par with the synthetic fungicide, Mancozeb 80WP. Corresponding to reduced disease severity, extract application increased grain yield by ca. 38-95% when compared with the negative control. Therefore, it could be concluded that, *S. molle* extracts can be considered as one of the alternative means of suppression of the negative effects of this fungus. However, screening of the active principle(s) of *S. molle* leaf extracts is subject to further research for effective utilization of this plant in crop protection.

Keywords: Disease incidence/severity, *In vitro*, *In vivo*, Plant extract, *Schinus molle*, Shallo, *Vicia faba*

1. Introduction

Ethiopia is considered to be the secondary center of diversity for faba bean (*Vicia faba* L.) (Asfaw *et al.,* 1994; Yohannes, 2000; Torres *et al*., 2006). The country is also among the major faba bean producing nations in the world ranking second next to China (FAO, 2006; Torres *et al.,* 2006). Faba bean is a valuable source of cheap protein for the poor that cannot afford to buy animal protein. Moreover, the crop naturally improves soil fertility through biological nitrogen fixation (Samuel *et al.,* 2008).

In spite of the above-mentioned advantages, faba bean yield is substantially lower in Ethiopia than the world's average, mainly due to negative impacts of various diseases. Among faba bean diseases, chocolate spot, caused by the fungus *Botrytis fabae* Sard, is the most widespread and highly destructive one causing a yield loss ranging roughly from 34 to 61% on susceptible cultivars in Ethiopia (ICARDA, 2006). The disease is found to be severely affecting yield wherever it occurs provided that a susceptible host and conducive environmental factors prevail (ICARDA 2006; Samuel *et al*., 2008).

So far, several disease management options have been devised for the prevention and management of diseases among which the use of synthetic fungicides is one. Even though synthetic protection chemicals appear to be effective, resistance development by the pathogens against them is a prevailing problem (Maggie *et al.,* 2006). Additionally, synthetic chemicals are hazardous to human health and non-target beneficial organisms, and can result in environmental pollution because of poor biodegradability (Isman, 2006).

Plant products (botanicals) are sought as alternative to synthetic chemicals as they are cheap and environmentally friendly. Plant-based chemicals are rich sources of secondary metabolites that have antimicrobial properties (Srivastava *et al*., 1996). They are easily biodegradable and hence considered safe to the environment and human health compared to the synthetic ones (Isman, 2006; Koul, 2008; Nerio *et al.,* 2010).

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Plant pathogens including *B. fabae* have been managed by the use of plant extracts. For example, complete inhibition of mycelial growth of *B. fabae* was recently reported by Hoda et al. (2016). Roman (2010) also had demonstrated growth inhibitory effects of crude extracts of 7 plant species (*Croton macrostachyus, Solanum incanum, Datura stramonium, Solanum marginatum, Calpurnia aurea, Clematis simensis,* and *C. hirsute*) on *B. fabae* under laboratory and greenhouse conditions. However, compared to the rich plant resources of Ethiopia, researches done so far on natural products that can effectively suppress fungal plant diseases like chocolate spot is limited. The objective of this study was, therefore, to investigate the efficacy of crude extracts of pepper tree (*Schinus molle*, Anacardiaceae) against *B. fabae*, a causative agent of chocolate spot of faba bean (*V. faba*, Fabaceae) both under laboratory and field conditions.

2.Materials and Methods

2.1. Test Plant Material and Pathogen

Fresh mature leaves of pepper tree (*Schinus molle*) were collected from around Sinana Agricultural Research Center (SARC) located at 07° N and 40° 10' E in Bale Zone, south-eastern Ethiopia. *Botrytis fabae* isolate was obtained from infected leaves of faba bean grown at SARC. Small portions of the infected faba bean leaves were cut from the advancing margin and dipped into 0.5% sodium hypochlorite for 2-3 min (Haggag *et al.*, 2006). Thereafter, the leaves were washed three times using sterilized distilled water. Then, the specimens were placed on the Faba bean dextrose agar (FDA) prepared as indicated in section 2.3 below and incubated at the temperature of 20±2 °C for seven days and then sub-cultured until a pure isolate was obtained. The isolate was identified by comparing with previous pure isolates from SARC and further cross checked with the reference from Barnett and Hunter (1982) for confirmation.

2.2. Preparation of Plant Extracts

Fresh leaf samples of *Schinus molle* were washed, air-dried in the laboratory and powdered using sterilized blender. The powder was then extracted using distilled water, methanol and ethanol separately. Stock extract was prepared by soaking leaf powder in distilled water, 99.8% methanol or 99.8% ethanol in 1:4 (w:v) ratio of leaf powder to the respective solvents left in the laboratory for 24 h with intermittent stirring using a sterile glass rod to ensure uniform soaking (Simeon *et al.,* 2008; Egigu *et al.*, 2010). The extract was then filtered first by using 4-fold cheese cloth (Wokocha and Okereke, 2005) and then using sterilized Whatman No. 1 filter paper. After centrifuging (at 6000 rpm for 15 min) the filtrate was serially diluted in the respective solvents to get 5, 10, 20 and 40% concentrations (Shovan *et al.*, 2008; Yeni, 2011). The extracts were then stored in air-tight bottles at 4 °C in a refrigerator until use in bioassay (Naduagu *et al.,* 2008; Prince and Prabakaran, 2011).

2.3. Preparation of Growth Medium

Faba bean dextrose agar (FDA) was prepared and used as a growth medium (Hanounik and Maliha, 1986; Haggag *et al.*, 2006). For this, coarsely chopped fresh faba bean leaf (400 g) was mixed with 1 L of tap water in a 1.5 L conical flask and autoclaved at 121 °C for 20 minutes. The sterilized leaf-water mixture was then filtered and the filtrate was mixed with agar medium (18 g) and dextrose (20 g). Thereafter, the mixture was heated along with uniform mixing and the volume was made up to 1 litre with tap water. This mixture was again autoclaved at 121 °C for 20 minutes, cooled down to about 40 °C and poured into Petri dishes (with 9 cm diameter) to serve as growth medium.

2.4. *In Vitro* Growth Test

Prior to pouring the growth medium into Petri dishes, two perpendicular lines that divided the Petri dishes into four equal sections were drawn at their bottom (Amadioha and Obi, 1999). The point of intersection of the lines represented the centre of the Petri dishes. Thereafter, the growth medium was poured into the Petri dishes with immediate addition of 2 ml of the different concentrations of the extract (Joseph *et al.,* 2008; Shovan *et al.,* 2008). Petri dishes that received distilled water or organic solvents represented negative control. Subsequently, a 4 mm diameter disk of mycelial pure culture was cut out using sterile cork borer and placed into the hole cut out from the solidified medium-extract mixture just at the point of intersection of the two lines drawn at the bottom of the plate (Yeni, 2011). The medium was modified by adding 0.2% (v/v) lactic acid to prevent bacterial contamination. The culture was incubated at 20±2°C for about 7 days and mycelial radial growth measurement was done when the growth in the control plates reached maximum. Fungi-toxicity of test extracts was then calculated in percentage mycelial growth inhibition using the formula indicated in Sundar *et al.* (1995) and Ahmed *et al.* (2002). The experiment was arranged in a completely randomized design (CRD) with three replications.

$$\%\text{Growth inhibition} = \frac{DC - DT}{DC} X100 \quad (1)$$

Where: DC = the average growth diameter from control (distilled water) plates, DT = the average growth diameter of fungal mycelia from extract treated plates

2.5. *In Vivo* Experiment

A faba bean variety, Shallo, which is released by Sinana Agricultural Research Center (Oromia Agricultural Research Institute, OARI) in 1999/00 was used for the field experiment. The trial was arranged in a factorial randomized complete block design (RCBD) with three replications. Plots of 2 m length and 1.2 m width were

used to cultivate faba bean. The plots had three rows of 2 m long spaced by about 0.4 m from one another. Adjacent plots were spaced by 1 m within a block, whereas adjacent blocks were spaced by 2 m. Plants were subjected to natural infection and disease development (Ogbebor and Adekunle, 2008; El-Sayed *et al.,* 2011). Thereafter, extracts were applied to the treatment plots soon after disease infection was noticed. Plots that received extracts were sprayed with 100% concentration of crude extracts (i.e., 1:4 w/v of plant material to solvent ratio) of individual solvents to sufficient wetting of the leaves. The extract concentration was not diluted as in the case of *in vitro* experiment deliberately to compensate for the probable loss of active principles under field conditions.

A standard synthetic fungicide, Mancozeb 80WP, was applied at the recommended rate (2.5 kg ha^{-1}) and distilled water and organic solvents were sprayed on control plots. Extract and fungicide application was done right after the disease symptom was seen in either one of the plots on the 61 days after planting and subsequent applications were made in a seven days interval up to 89 DAP. Prior to extract application, potential phytotoxic effects of the extracts and solvents were also evaluated by applying onto sample leaves of the crop to inspect the development of leaf injury symptoms for a week time.

For collection of agronomic data including number of aborted flowers $plant^{-1}$, number of tillers m^{-2}, number of pods $plant^{-1}$, number of seeds pod^{-1}, hundred seed weight (HSW) in gm and grain yield (kg ha^{-1}), five plants from middle row were randomly taken and marked with colored threads for identification. Disease incidence was assessed by counting plants showing symptoms of the disease and expressed in percent (%) infection. Disease severity was assessed as the proportion of plant structure affected by the disease from the five randomly tagged plants in the middle row. Disease severity was recorded using 1-9 scale: where, 1= no disease symptoms or very small specks; 3= few small discrete lesions; 5= some coalesced lesions with some defoliation; 7= large coalesced sporulating lesions, 50% defoliation and some dead plant; and 9= extensive lesions on leaves, stems and pods, severe defoliation, heavy sporulation, stem girdling, blackening and death of more than 80% of plants (Bernier *et al.,* 1993). Disease severity data was then converted to Percent Severity Index (PSI) using the following formula developed by Wheeler (1969) and presented as:

$$PSI = \frac{Snr}{NprXMss} X100 \quad (2)$$

Where: PSI = Percent severity index, Snr=Sum of numerical ratings, Npr = Number of plants scored and Mss = the maximum scale of the disease

2.6. Data Analysis

Statistical data analyses were conducted with the statistical packages of the computer software SPSS for Windows 16.0 (SPSS; Chicago, IL, USA). Data were first checked for normality of distribution and logarithmically transformed as necessary. Analysis of variance (ANOVA) was used to analyze data and the treatments means were separated by the least significant difference (LSD) and differences between means were considered to be statistically significant at $p \leq 0.05$ (Gomez and Gomez, 1984). Regression analysis was done to reveal the efficacy of extract with increasing concentration.

3. Results

3.1. Efficacy of *S. molle* Extracts on *in Vitro* Growth of *Botrytis fabae*

Both organic (methanol and ethanol) and aqueous extracts of *S. molle* at all concentration levels, including 10, 20 and 40% (i.e. with the exception of ethanolic extract at 5% level), highly and significantly ($p \leq 0.01$) reduced mycelial growth of *B. fabae* when compared to the control (distilled water: data for the control is not presented as it is zero). Growth inhibitory effects of extracts increased with extract concentration though no significant difference was observed between 5 and 10% concentrations of aqueous extracts (Figure 1A, B and C). Regression analysis showed that each unit increase in methanol, ethanol and aqueous extracts concentrations increased their respective efficacies by 1.6, 1.3 and 1.4%, respectively, where the coefficients of determination (R^2) were explained by over 80%.

There was significant ($p \leq 0.05$) difference among the different extracts due to the solvents in inhibiting growth of *B. fabae* at each concentration level (Figure 2). At the two lower concentrations (i.e. 5 and 10%), aqueous extracts resulted in maximum growth inhibition followed by methanolic and ethanolic extracts. However, at the two upper concentration levels, methanolic extracts showed the highest efficacy, followed by aqueous and ethanolic extracts.

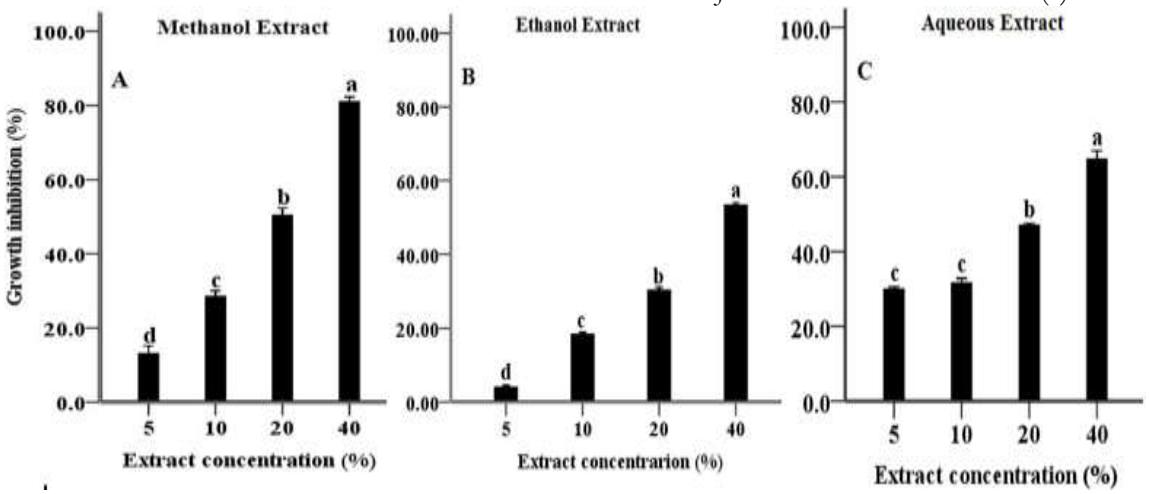

Figure 1. *In vitro* growth inhibitory effect of the three solvents' extracts of *S. molle* on *B. fabae*. Values are Mean ± S.E., n = 3. Error bars with same letter are not significantly different, whereas those with different letters are significantly different at $P<0.05$.

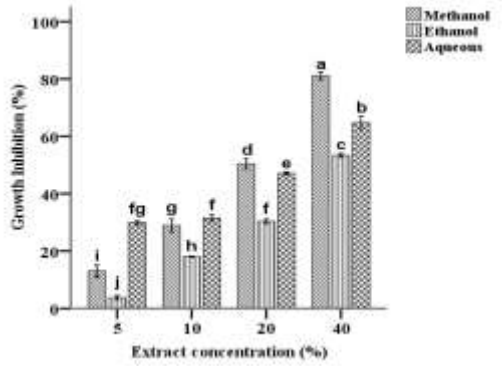

Figure 2. Comparison of the three solvents' extracts of *S. molle* on *B. fabae* mycelial *in vitro* growth. Values are Mean ± S.E., n =3. Error bars with same letter are not significantly different, whereas those with different letters are significantly different at $P < 0.05$.

3.2. Effects of *S. molle* Extracts on the Disease Incidence and Severity Caused by *B. fabae* Under Field Conditions

Treatment application with extracts was done on the 61st day after planting (DAP) immediately on the onset of infection and continued on a weekly basis up to 89 DAP at which final percent incidence and disease severity were assessed. On the 89 DAP, disease incidence was 100% in plots sprayed with distilled water (control). This value was significantly decreased in plots sprayed with synthetic fungicide and *S. molle* extracts. Disease incidence reduction was highest in methanol extract and synthetic fungicide sprayed plots, followed by ethanol and aqueous extracts sprayed plots (Figure 4).

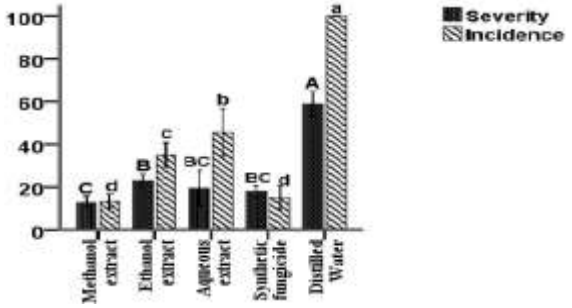

Figure- 3. Percent disease severity (black bar) and percent disease incidence (striped bar) caused by *B. fabae* on *V. faba* treated with *S. molle* extracts under field conditions. Values are Mean ± S.E., n=3. Error bars with same letter are not significantly different, whereas those with different letters are significantly different at $P < 0.05$. Capital letters compare disease severity between treatments, whereas small letters compare disease incidence between treatments.

Disease severity was significantly reduced by all types of *S. molle* extracts and synthetic fungicide compared to the control. The synthetic fungicide and all types of *S. molle* extracts showed a similar efficacy in disease severity reduction (Figure 3). Disease severity kept advancing throughout the experimental period in control plot, but halted at about constant level (<25%) in extract and synthetic fungicide-treated plots (Figure 4).

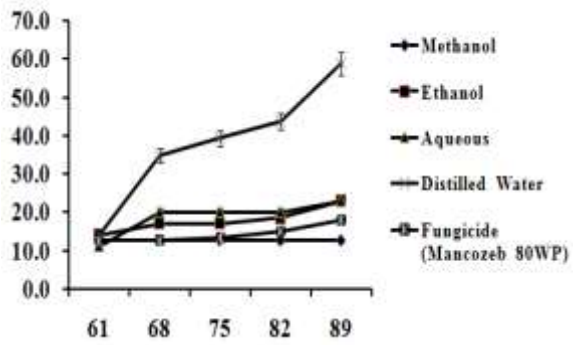

Figure 4. Disease severity pattern as assessed in a weekly basis of extract application.

3.3. Effect of Crude Extracts of *S. molle* on the Yield and Yield Components of Faba Bean

No significant difference was observed among the treatments and the negative control with regard to the number of pods per plant, number of seeds per pod and hundred seed weight (HSW). Except that of ethanol, all types of extracts of *S. molle* significantly reduced flower abortion when compared with that of the negative control. Moreover, the number of tillers counted from plots and pods per plant in plots treated with all types of extracts of *S. molle* were significantly higher than that of the control plot. Grain yield obtained from plots treated with extracts and synthetic fungicide significantly increased when compared with that of the negative control (Table 1). Percent infection of faba bean by *B. fabae* and chocolate spot disease severity were significantly ($p < 0.01$) lower in plots sprayed by *S. molle* leaf extracts than the negative control plots (Figure 3). Assessment of disease severity on a weekly basis revealed that symptoms were consistently advancing in negative control plot than in extract and fungicide sprayed plots (Figure 4).

Table 1. Effect of crude extracts of *S. molle* on yield and yield components of faba bean.

Treatments	No. of aborted flowers per plant	No. of tillers/m²	No. of pods/plant	No. of seeds/pod	HSW (g)	Grain yield (kg/ha)
Methanol extract	12.6±1.3c	22.0±0.2ab	20.2±1.6a	3.2±0.0a	56.5±1.3a	3739.3±43.3a
Ethanol extract	21.0±3.5ab	17.6±1.8b	22.0±1.2a	3.3±0.1a	54.9±0.5a	4236.9±15.2a
Aqueous extract	14.0±3.5bc	13.0±0.9c	16.1±0.2a	3.1±0.1a	58.2±0.6a	2995.7±12.1a
Fungicide (Mancozeb 80 WP)	11.8±3.6bc	17.3±0.8b	18.1±2.7a	3.3±0.1a	60.8±1.5a	3404.0±22.7a
Distilled water	23.9±3.2 a	8.3±0.9d	11.0±5.3b	3.2±0.1a	60.6±3.3a	2167.1±39.4b

Note: Means with different letters within a column are significantly different, whereas means with the same letter within a column are not significantly different from each other at P < 0.05. Values are Mean ± S.E., n=3; HSW= hundred seed weight.

4. Discussion

4.1. Growth Inhibition of *B. fabae* due to *S. molle* Extracts

Results of the *in vitro* experiment showed that all *S. molle* leaf extracts were considerably efficacious in inhibiting the growth of *B. fabae* in a concentration-dependent manner. Some organic compounds used as extraction solvents may have growth inhibitory effect on microbes. In this experiment, both methanol and ethanol that were used as solvents of extraction were used as negative control and compared with that of distilled water. The result showed that the two organic solvents had no negative effect on *B. fabae* growth, suggesting the observed growth inhibitory effects of the extracts were entirely attributed to the extract constituents.

Similar to the results of this study, Ibrahim and Al-Naser (2014) reported the growth inhibitory effect of fruit extracts of *S. molle* on *Botrytis cinerea* and revealed the presence of terpenoids such as α-pinene, β-pinene, α--phellandrene β-phellandrene and limonene in the extracts. Recently, Muhd *et al.* (2015) had also reported that *S. molle* leaf extracts possess secondary compounds such as terpenoids (monoterpenes and sesquiterpenes), which are reported to have antimicrobial properties. The antifungal effects of *S. molle* leaf essential oil had also been reported on other fungal species, such as *Aspergillus ochraceus*, *Aspergillus parasiticus*, *Fusarium culmorum* and *Alternaria alternata* (Gundidza, 1993). Though all solvents' extracts were effective to suppress growth at all concentration levels, that of ethanol performed less compared to methanol and aqueous extracts. Especially, its performance at 5% concentration did not vary from the control. Moreover, aqueous extracts were superior to both methanol and ethanol at 5 and 10% while methanol extract was superior to aqueous and ethanol at 20 and 40% extract concentration. This shows that solvents of different polarity have different potential of extracting and yielding compounds of varying bioactivity (Egigu *et al.*, 2010).

4.2. *S. molle* Extracts Suppress Chocolate Spot Disease of Faba Bean

Under the field condition, infection of faba bean by *B. fabae* and the accompanying disease severity were greatly reduced in plots sprayed with *S. molle* extracts and the positive control synthetic fungicide, Mancozeb 80WP. Last assessment made on the 89 DAP showed that disease incidence and severity were at ca. 100 and 60%, respectively. Methanolic extract was on a par with synthetic fungicide in disease incidence reduction,

followed by ethanolic and aqueous extracts. Assessments made at a seven days' interval showed that disease severity advanced in control plots, but progress was effectively halted and kept below 25% by *S. molle* extracts. This shows that leaf extracts from *S. molle* could also suppress chocolate spot severity under field conditions as they suppress the growth of *B. fabae* under laboratory conditions. Similar to its efficacy under laboratory conditions, methanolic extracts appeared to perform better than ethanolic and aqueous extracts under field conditions, suggesting the stability of its extract constituents under field conditions. Some compounds are easily degradable under field conditions due to ultraviolet light and/or extreme temperatures (Schmutterer, 1990).

The rate of disease severity showed a sharp increase in control plots when one week after the onset of chocolate spot measured. Severity kept on increasing afterwards between 68 and 82 DAP, but the rate of increase was slower. This shows that naturally faba bean produces defense chemicals to fight back pathogens once attacked. Plants produce novel defense chemicals and/or increase the production of constitutive defense chemicals upon attack by pathogens and herbivores (Turlings *et al.*, 1990; Loughrin *et al.*, 1994; McCall *et al.*, 1994; Dudareva *et al.*, 2006). The fact that disease severity regained its momentum between 82 and 89 DAP in the control plots shows that production of defense chemicals may be age dependent in which plants invest less resources to defense chemicals than other purposes, reproduction, for example. Time series measurement is worth taking in the future to elucidate the pattern of defense compounds (secondary compounds) production specific to faba bean.

Corresponding to disease severity reduction, seed yield, number of tillers plot^{-1}, number of pods plant^{-1} and flower retention were positively affected by extract application. Interestingly, for example, estimate of grain yield obtained from extract sprayed plots increased in an order of 38 to 95% when compared to the negative control plots. This result is in agreement with the report of ICARDA (2006) which mentions that *Botrytis fabae* can cause a yield loss ranging roughly from 34 to 61% on susceptible faba bean cultivars in Ethiopia.

5. Conclusion

The results from the *in vitro* and *in vivo* experiments have demonstrated that *S. molle* extracts have negative impacts on *B. fabae*. Compared to the control, mycelial growth was significantly reduced by extracts under lab conditions. Results of field experiment showed significant reduction in disease severity that helped to increase grain yield in plots sprayed with extracts when compared with that of negative control. This indicates that *S. molle* extracts possess bioactive compounds against *B. fabae,* which needs safety evaluation of crude extracts not to damage non-target organisms and screening of active principle(s) for use by farmers.

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7. References

Ahmed M. F., Khalequzzaman, K. M., Islam M. N., Anam, M. K. and Islam, M. T. 2002. Effect of Plant Extracts against *Bipolaris oryzae* of rice under *in vitro* conditions. *Pakistan Journal of Biological Sciences,* 5: 442-445.

Amadioha, A. C. and Obi, V. I. 1999. Control of anthracnose disease of cowpea by *Cymbopogoncitratus* and *Ocimum gratissimum*. *Acta Phytopathologica Entomol. Hungerica,* 34: 85-89.

Asfaw, T., Geletu, B., Saxena, H. C. and Solh, M. B. (Eds.). 1994. Cool-season Food Legumes of Ethiopia. pp. 1- 18. *In*: Proceedings of the First National Cool-Season Food Legumes Review Conference, 16-20 December 1993, Addis Ababa, Ethiopia. *ICARDA/Institute of Agricultural Research*. ICARDA: Aleppo, Syria. Vii + 440 pp.

Bernier, C. C., Hanounik, S. B., Hussein, M. M. and Mohamed, H. A. 1993. Field manual of common faba bean diseases in the Nile Valley. International Center for Agricultural Research in the Dry Areas (ICARDA) Information Bulletin No. 3.

Dudareva, N. Negre, F. Nagegowda, D. A. and Orlova, I. 2006. Plant volatiles: recent advances and future perspectives. *Critical Review in Plant Science,* 25: 417-440

Egigu, M. C., Ibrahim, M. A., Yahya, A. and Holopainen, J. K. 2010. Yeheb (*Cordeauxia edulis*) extract deters feeding and oviposition of *Plutella xylostella* and attracts its natural enemy. *Biocontrol*, 55: 613-624.

El-Sayed, A. S., Rania, Z., El, S. and Ismail, A. I. 2011. Fungicidal management of chocolate spot of faba bean and assessment of yield losses due to the disease. *Annals of Agricultural Sciences,* 56: 27-35.

FAO. 2006. The state of food insecurity in the world: Eradicating world hunger – taking stock ten years after the World Food Summit. ftp://ftp.fao.org/docrep/fao/009/a0750e/a0750e00.pdf. (accessed March, 2016)

Gomez, K. A. and Gomez, A. A. 1984. Statistical Procedures for Agricultural Research, 2nd Edn. John Wiley and Sons Inc. New York, Singapore. 680 pp.

Gundidza, M. 1993 Antimicrobial activity of essential oil from *Schinus molle* Linn. Central *Africa Journal of Medicine*, 39: 231-234.

Haggag, W. M., Kansoh, A. L. and Aly, A. M. 2006. Proteases from *Talaromyces flavus* and *Trichoderma harzianum*: Purification, characterization and

antifungal activity against brown spot disease on faba bean. *Plant Pathology Bulletin,* 15: 231-239.

Hanounik, S. B. and Maliha, N. 1986. Horizontal and Vertical Resistance in *Vicia faba* to Chocolate Spot Caused by *Botrytis fabae*. *Plant Disease*, 70: 770-773.

Hoda, M. S., Migahed, F. F. and Nofel, A. M. 2016. Role of Plant Extract and Essential Oil of *Eugenia caryophyllus* (Spreng.) in Controlling Chocolate Spot of *Vicia faba* L. *Egyptian Journal of Biological Pest Control*, 26: 469-475.

Ibrahim, B. and Zakaria, Al–Naser. 2014. Analysis of fruits extractions of *Schinus molle* and the efficacy in inhibition of growth of the fungi in laboratory. *International Journal* of *ChemTech Research* 6: 2799-2806.

ICARDA (International Center for Agricultural Research in the Dry Areas). 2006. Technology Generations and Dissemination for Sustainable Production of Cereals and Cool Season Legumes. Aleppo, Syria. 256 pp.

Isman, M. B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology,* 51: 45-66.

Joseph, B., Dar, M. A. and Kumar, V. 2008. Bioefficacy of Plant Extracts to Control *Fusarium solani* F. Speg. Melongenae Incitant of Brinjal Wilt. *Global Journal of Biotechnology and Biochemistry* 3: 56-59.

Koul, O. 2008. Phytochemicals and insect control: an antifeedant approach. *Critical Review of Plant Science* 27: 1-24.

Loughrin, J. H., Manukian, A., Heath, R. R., Turlings, T.C.J. and Tumlinson, J.H. 1994. Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plant. Proceedings of the National Academy of Sciences USA 91: 11836-11840.

Maggie, E. M. H., Abd El-Rahman, S., El-Abdasi, L.H. and Mikhail, M.S. 2006. Inducing resistance against faba bean chocolate spot disease. *Egyptian Journal of Phytopathology*, 34: 69-79.

McCall, P.J., Turlings, T.C.J., Loughrin, J., Proveaux, A.T. and Tumlinson, J. H.1994. Herbivore induced volatile emissions from cotton (*Gossypium hirsutum* L.) seedlings. *Journal of Chemical Ecology*, 20: 3039-3050.

Muhd, P. S. D., Cuelho, C. H. F., Brondani, J. C. and Manfron, C. M. P. 2015. Chemical composition of the *S. molle* L. essential oil and their biological activities. *Revista Cubana de Farmacia,* 49: 132-143.

Naduagu, C., Ekefan, E.J. and Nwankiti, A. O. 2008. Effect of Some Crude Plant Extracts on Growth of *Colletotrichum capsici* (Synd) Butler and Bisby Causal Agent of Pepper Anthracnose. *Journal of Applied Biosciences,* 6: 184-190.

Nerio, L. S., Olivero-Verbel, J. and Stashenko, E. 2010. Repellent activity of essential oils: A review. *Bioresource Technology*, 101: 372-378.

Ogbebor, O. N. and Adenkunle, A. T. 2008. Inhibition of *Drechslera heveae* (Petch) M. B. Ellis, causal organism of bird's eye spot disease of rubber (*Hevea brasiliensis* Muell Arg.) using plant extracts. *African Journal of General Agriculture,* 4: 19-26.

Prince, L. and Prabakaran, P. 2011. Antifungal activity of medicinal plants against plant pathogenic fungus *Colletotrichum falcatum*. *Asian Journal of Plant Science and Research*, 1: 84-87.

Roman, M. 2010. Evaluation of antifungal activity of plant extracts against chocolate spot disease (*Botrytis fabae*) on Faba bean. M.Sc. thesis, Addis Ababa University.

Samuel, S., Ahmed, S., Fininsa, C., Abang, M. M. and Sakhuja, P. K. 2008. Survey of chocolate spot (*Botrytis fabae*) disease of faba bean (*Vicia faba* L.) and assessment of factors influencing disease epidemics in northern Ethiopia. *Crop Protection* 27: 1457-1463.

Schmutterer, H. 1990. Properties and Potential of Natural Pesticides from the Neem Tree, *Azadirachta inidica*. *Annual Review of Entomology,* 35: 271-97.

Shovan, L. R., Bhuiyan, M. K. A., Begum, J. A. and Pervez, Z. 2008. *In vitro* Control of *Colletotrichum dematium* Causing Anthracnose of Soybean by Fungicides, Plant Extracts and *Trichoderma harzianum*. *International Journal of Sustainable Crop Production,* 3: 10-17.

Simeon, A., Materechera, M. and Hae, E. 2008. Potential of Aqueous Extracts from Parts of the Pepper Tree (*Schinus molle* L.) to Affect Emergence and Seedling Development of Wheat (*Triticum sativa* L.) and Weeds in a Manure Amended Soil. *The Open Agriculture Journal*, 2: 99-104.

Srivastava, J., Lambert, J. and Vietmeyer, N. 1996. Medicinal plants: An expanding role in development. *World Bank Technical Paper*, No 320.

Sundar, A. R., Das, N. D. and Krishnaveni, D. 1995. *In vitro* Antagonism of *Trichoderma* spp. against Two Fungal Pathogens of Castor. *Indian Journal of Plant Protection,* 23: 152-155.

Torres, A. M., Roman, B., Avila, C. M., Satovic, Z., Rubiales, D., Sillero, J. C., Cubero, J. I. and Moreno, M. T. 2006. Faba bean breeding for resistance against biotic stresses: towards application of marker technology. *Euphytica,* 147: 67-80.

Turlings, T. C. J., Tumlinson, J. H. and Lewis, W. J. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, 250: 1251-1253.

Wheeler, J. B. 1969. An introduction to plant diseases. Wiley and Sons, London, P. 347.

Wokocha, R. C. and Okereke, V. C. 2005. Fungitoxic activity of extracts of some medicinal plants on *Sclerotium rolfsii,* causal organism of the basal stem rot diseases of tomato. *Nigerian Journal of Plant Protection,* 22: 106-110.

Yeni, I. J. 2011. Evaluation of Antifungal Effects of Extracts of *Allium sativum* and *Nicotiana tobacum* against Soft Rot of Yam (*Dioscorea alata*). *Researcher,* 3: 1-5.

Yohannes, D. 2000. Faba Bean (*Vicia faba*) in Ethiopia. Institute of Biodiversity, Conservation and Research (IBCR). Addis Ababa, Ethiopia. 43 p.