Efficacy of Natural Oils from Neem and Eucalyptus against Chocolate Spot (*Botrytis fabae)* of Faba bean (*Vicia faba* L.)

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ABSTRACT: *This experiment was conducted in the Department of Dry land Crop and Horticultural Sciences green house unit, Mekelle University with the main objective to evaluate the efficacy of natural oils extracted from neem (N) and eucalyptus (E) plants against chocolate spot. The natural oils were prepared at C1=3000ppm, C2= 4000ppm and C3=5000ppm concentration levels from each Neem and Eucalyptus. Three Faba bean varieties viz: hachalu (V1), moti (V2) and gora (V3) were grown at Mekelle University greenhouse arranged using CRD experimental design with factorial experiment replicated trice. Data on disease incidence, inhibition, severity and number of aborted flowers per plant, number of diseased leaves per plant and number of pods per plant were recorded. Maximum inhibition was observed from NV2C3 (72%) followed by NV3C3 (69%), EV3C3 (68%) and NV1C2 (64%) whereas minimum inhibition was scored in NV1C3 (23%). Maximum incidence was observed in EV1C1 (27%) while minimum incidence was recorded from EV1C3 (19%). Maximum severity (55%) was recorded from EV1C1 and EV3C1 followed by EV3C2 (53%) whereas the least severity of 25% was recorded from NV1C2, NV1C3, NV2C3 and NV3C3. The statistical analysis confirmed that the varieties had responded to the concentration differently and shown significant positive effect on the parameters studied. Therefore, chocolate spot (Botrytis fabae) disease of faba bean (Vicia faba L.) can be inhibited by natural oils extracted from Neem and Eucalyptus.*

Key Words: *Chocolate spot, Faba bean and Natural oils*

Introduction

Faba bean (*Vicia faba* L.*) is* a food legume which has been domesticated most probably in the late Neolithic period (Metayer, 2004). It is mainly used as food and feed in different forms like soup, bread and roasted and animal feed etc. Ethiopia is the second highest faba bean producer next to China (FAOSTAT, 2009 and CSA, 2013) and it occupies the largest share and production of pulses grown in the world (CSA, 2013). Even though Ethiopia is the world's second largest producer of faba bean, its share is only 6.96% of world production and 40.5% within Africa (MoA, 2011 and CSA, 2013) and is declined from 4.8 million ha in 1961 to 2.4 in 2008 with the reduction in production from 4.8 tons per hectares to 4.4 tons per hectares (FAO, 2008). The reasons for the decline in production are susceptibility to biotic and abiotic factors (Mussa *et al*., 2008, Sillero *et al.,* 2010 and Link *et al.,* 2010). Among the biotic stresses, chocolate spot is highly contributed to the low productivity of the crop **(**El-Banoby *et al*., 2013 and Wolde *et al.,* 2015).

The commonly used practice to control chocolate spot disease in different faba bean producing countries was chemical pesticides (Sahile *et al*., 2009; Fekadu, 2014). However, these chemical pesticides are not eco-friendly and are harmful to living organisms (Sitara *et al*., 2011; Kariuki *et al*., 2014; Wolde *et al.*, 2015**)**. With the growing demand for environmentally sound strategies in the control of pests, the development of alternative pesticides with minimal ecological hazards has now become an imperative need (Dubey *et al*., 2010). Natural pesticides are active substances imitated from plants used for pest treatment. Many plant extracts show a broad spectrum of activity against several pests and pose little threat to human health and the environment (Damalas, 2011). Of these plants, Neem and Eucalyptus have been used as antimicrobial activities such as insects, fungi and bacteria (Yang *et al*., 2004; Maciel *et al*., 2010). However, information on the efficacy of those natural oils against chocolate spot disease of faba bean is limited. Therefore, this study was aimed to evaluate the effectiveness of natural oils extracted from neem and eucalyptus against chocolate spot disease of faba bean.

Materials and Methods

The experiment was conducted in Mekelle University's green house unit. Two plant species such as neem (*Azadirachta indica) and* eucalyptus *(Eucalyptus globules)* were used for the extraction of oils to treat

chocolate spot disease of faba bean. From each plant species, fresh leaves were collected from three districts (Ganta afeshum, Hawzen and Atsbi-wenberta) of eastern zone of Tigray, Ethiopia and the samples were taken to Mekelle University organic chemistry laboratory for oil extraction and to test for their efficiency to control faba bean chocolate spot under green house conditions.

Three faba bean varieties such as Hachalu (V1), Moti (V2) and Gora (V3) that were procured from Tigray Agricultural Research Centre were grown in polyethylene pots (40 cm, five plants/pot) arranged using complete randomized design (CRD) in factorial experiment replicated trice. The distance between the plots was 1.5m. The natural oils were prepared at C1=3000 ppm, C2= 4000 ppm and C3=5000 ppm concentration levels from each Neem and eucalyptus. Culture filtrates of *Botrytis fabae* was prepared according to the method described by Mukherjee *et al.,* (1995). The treatments were inoculated with spore suspension of *Botrytis fabae* after 25 days of planting. After five days of inoculation, the plants were sprayed with the natural oils as per the combinations. This was done according to the accepted methodology developed by Stoddard and Herath (2001).

Data on disease severity was obtained using the devised scale of Bernier *et al.,* (1993). The Disease severity was calculated as: 1= No disease symptoms or very small specks; 3 = Few small disease lesions; 5 = some coalesced lesions, with some defoliation; 7 = Large coalesced sporulating lesions; 9 = Extensive, heavy sporulation, stem girdling, blackening and death of more than 80 % of plants.

Means of the severity grades obtained were converted in to Percentage Severity Index (PSI) Wheeler, (1969).

$$\text{Disease severity (DS)}\,\% = \frac{\Sigma\,(\text{NPC}\times\text{CR})}{\text{NIP}\times\text{MSC}}\times 100$$

Where*:*

NPC = No. of plants in each class rate
CR = Class rate
NIP = No. of infected plants
MSC = Maximum severity class rate

The inhibition percent of *Botrytis fabae* was calculated using the formula:

$$\text{Reduction \%} = \left[\frac{C-T}{C}\ X100\right]$$

Where: C = fungal growth area of *Botrytis fabae* in control treatment.
T = fungal growth area of *Botrytis fabae* in presence of antagonist

Disease incidence was obtained as the proportion of infected plants expressed as percentage of the total plants assessed, number of diseased leaves per plant was be obtained by counting the number of leaves affected by Botrytis fabae, number of aborted flowers per plant was obtained by subtracting the number of flowers which bear pod from the total number of flowers beard by the plant, number of pods per plant: this was obtained by counting the number of pods in each plan

Data Analysis

The collected data was subjected to analysis of variance with genstat v. 16 using PROC MIXED procedure. The means between the treatments were compared with least Significance Difference (LSD) at 5% probability level. Before running the combined analysis, Bartlett's test was used to satisfy the assumption of homogeneity of variances.

Results and Discussion

Inhibition of chocolate spot disease of faba bean as treated by Neem and Eucalyptus oils

The computed result revealed, natural oils extracted from neem and eucalyptus can inhibit chocolate spot disease of faba bean. Furthermore, as the concentration of oils increase the percent of inhibition also increases. The highest inhibition percentage was 72% which was scored on NV2C3 followed by NV3C3 (69%), EV3C3 (68%) and NV1C2 (64%) whereas minimum inhibition was scored in NV1C3 (23%). It was noticed that the varieties also responded to the concentration differently. Inline to the present finding, Da-Costa *et al*. (2010) utilized Neem extract to inhibit fungal growth (*i.e.* mycelia dry weight, diameter of colony and growth rate) of *Aspergillus flavus* on solid media at concentrations from 0.5 to 5.0% v/v and significantly increased sporulation in the same conditions. Similarly**,** Alabi *et al.* (2005) studied antimicrobial potential of *E. globulus* leaf extract against fungal disease of cowpea and found that it reduced the disease infection to about 2.4% compared to control (29%) and Satish *et al.,* (2007) studied the efficacy

of aqueous and organic solvents extracts of *E. globules* against seed borne fungal pathogens caused by different species of *Aspergillus* and found that *E. globules* among other plant species was highly effective against a number of species of the test pathogen.

Incidence of chocolate spot disease of faba bean as treated by Neem and Eucalyptus oil

In the current study, maximum incidence of chocolate spot disease was observed in EV1C1 (27%) followed by NV1C1 (24%) while minimum incidence was noticed on EV1C3 (19%). This clearly shows that, Eucalyptus oil treatment can reduce the incidence of chocolate spot of faba bean. This result is in conformity with the findings of Abera *et al.,* (2011) in their evaluation of botanical extracts against coffee berry disease causing fungus, *C. kahawae* and *E. globules* ethanol extract controlled the mycelial growth better than it did with its aqueous extract. The authors confirmed the percent inhibition of *C. kahawae* and *E. globules was* 64% and 57%, respectively as compared to the untreated control. Similar to the present finding, Deepak *et al.,* (2007) revealed aqueous extract of *E. globulus* leaves was proved to completely inhibit zoosporangium formation of the fungus Sclerospora *graminicola* which is the causative agent of downy mildew of Pearl millet.

Severity of chocolate spot disease of faba bean as treated by Neem and Eucalyptus oil

In the present study, the mean maximum chocolate spot severity of 55% was recorded from EV1C1 and EV3C1 followed by EV3C2 (53%) whereas the least severity score of 25% was recorded from NV1C2, NV1C3, NV2C3 and NV3C3. This experiment revealed, Neem oil exhibited best efficacy to reduce the severity of chocolate spot disease of faba bean than Eucalyptus. Similar findings were also reported by El-Banoby (2013) who studied biological control of chocolate spot disease of faba bean using some bioagents under greenhouse conditions using culture filtrate of *T. harzianum*, *A. qusisqualis* and *T. viride* at 50% concentration and reduced the disease severity by 30.3, 35.0 and 36.8% after five days treatment.

Analysis of variance for yield related traits

The result of analysis of variance computed on traits as described in Table 1 showed that there were significant mean differences due to natural oils, varieties and combination of natural oils with varieties.

Table1. ANOVA mean squares for different traits of faba bean

Source of variation	df	NAFP	NDLP	NPP	NSPO
Rep	2	0.08333	2.5957	2.0864	0.0123
Natural oil	8	11.84722**	12.9035**	1.679**	1.0401**
Var	2	55.36111**	232.2623**	21.0864**	0.6049
Var.x Nat.oil	16	2.63889**	5.5679**	2.4614**	1.2022**
Error	52	0.2265	0.679	0.607	0.3749

Where: NAFP= number of aborted flowers per plant, NDLP= number of diseased leaves per plant, NPP= number of pods per plant, NSPO= number of seeds per pod

The experiment revealed that application of the plant extracts improved some agronomic traits, such as number of aborted flowers, number of diseased leaves per plant and number of seeds per pod (Table 2). Similarly, Alabi *et al*. (2005) work revealed that use of *E. globulus* had positive effect on the agronomic traits of cowpea. Number of pods per plant, hundred seeds weight and yield were improved. This finding is consistent with the work of Locke (1995) who reported 2 to 10% neem oil has been completely controlled *A. alternata, A. niger* and *F. oxysporum* in field experiment.

Table 2. Efficacy of natural oils from Neem and Eucalyptus against chocolate spot of faba bean

TRT	NAFP	NDLP	NPP	NSPO
E1V1	7.0c	16.0 a	6.7a	1.7ed
E1V2	5.0e	12.0efd	3.7gefh	3.0ab
E1V3	4.0fg	16.0a	5.0bcd	3.0ab
E2V1	8.0b	9.0 ih	5.0bcd	1.0e
E2V2	4.0e	11.7ef	2.3i	3.3a
E2V3	3.0h	15.7a	3.7gefh	2.0cd

E3V1	6.0d	8.7ih	6.0ab	3.0ab
E3V2	4.0fg	8.7 ih	3.0hi	3.0ab
E3V3	2.7h	13.3bcd	5.3bc	3.0ab
N1V1	7.7 b	8.3ij	4.0efcd	2.3bcd
N1V2	8.0 b	11.8ef	3.3gh	1.7ed
N1V3	4.0fg	14.0bc	5.0bcd	3.0ab
N2V1	7.8b	7.2ih	5.0bcd	2.0cd
N2V2	9.0a	11.7ef	3.0hi	2.0cd
N2V3	5.0e	12.6ecd	4.0gefhd	2.0cd
N3V1	6.0 d	5.0k	6.0ab	2.7abc
N3V2	5.0e	9.0ih	2.3i	1.7ed
N3V3	4.0fg	10.0gh	5.0bcd	2.0cd
G mean	**5.10**	**10.48**	**4.34**	**2.5**
CV	**4.4**	**6.4**	**14.0**	**15.3**
LSD(0.05)	**0.371**	**1.1125**	**0.9946**	**0.6142**

Where: NAFP= number of aborted flowers per plant, NDLP= number of diseased leaves per plant, NPP= number of pods per plant, NSPO= number of seeds per pod

Conclusion

From the present investigation, it can be concluded that chocolate spot (*Botrytis fabae)* disease of faba bean (*Vicia faba* L.) can be inhibited by considerable degree as treated by natural oils extracted from plants of Neem and Eucalyptus.

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