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ANTIVIRAL ACTIVITY OF CLOVE AND BITTER ALMOND OILSAGAINST*BEAN YELLOW MOSAIC VIRUS* (BYMV) INFECTING FABA BEAN IN IRAQ

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ABSTRACT

This study was initiated to test the antiviral effect oftwo essential oils against *Bean yellow mosaic virus*infectingfaba bean plants.Clove and bitter almond oils were selected to test their activities.BYMV source was obtained from infected faba been seeds. Symptomatic plants grown were tested for BYMV infection using double antibody sandwich-enzyme linked immunoassay (DAS-ELISA). BYMV infected plants were sprayed with 5% clove oil and bitter almond oil (20 replicates each) twice within 2 weeks intervals. Results revealed symptomatic faba bean plants, for both treatments, were improved when symptoms were faded or disappeared. Faba bean plants treated with clove oilCO showed lower virus concentration more than bitter almond oil BAO when tested by DAS-ELISA when means of absorption values were 0.72 and 1.32 respectively compared to 2.92 for infected plantcontrol IPC. Growth parameters showed plants treated with BAO Our results suggest that essential oil treatmentscould be a promising method to control BYMV infectingfaba bean in Iraq.

KEYWORD: potyviruses, *Viciafaba*, virus disease, induced resistance, broad bean, Eugenol.

INTRODUCTION

Faba bean or broad bean (*Viciafaba*) is a leguminous crop grown in Iraq due to its economic importance. Dry and freshseeds of faba bean are used for domestic consumption and a source of income as they areused for cooking traditional meals in many restaurants in Iraq. The estimated yield of faba bean seeds was 6060 tons according to FAO statistics in (2014).Many pestshave been reported to attack faba bean in Iraq, including viruses, causing serious losses

(Al-Kuwaity, 2013). About 50 different viruses have been reported to infect faba bean worldwide (Makkouket al., 2012), whereas seven viruses only have been reported in Iraq so far (El-Muadhidiet al., 2001). *Bean yellow mosaic virus* (BYMV) belongs to the genus *Potyvirus* the family *Potyviridae* according to ICTV in (2015). It was reported for the first time to infect faba bean in Iraq in 1983 and becamethe most highly incident by 80-100% among other faba bean viruses since then in Iraq(El-Muadhidiet al., 2001; Al-Kuwaity, 2013). BYMV is an aphid transmissible in a non-persistent manner and through faba bean seeds by up to 17% (El-Muadhidi et al., 2001; Mali et al., 2003; Makkouk et al., 2012).Essential oils are hydrophobic, volatile and aromatic compounds provide scent, flavor or characteristic odor to a plant. They are volatile secondary metabolites and by-products of plant metabolism (Arshadet al., 2014).Due to their antimicrobial activity, essential oils from different plants were applied against plant pathogens including several viruses (Koulet al., 2008;Bezić et al., 2011; Arshad et al., 2014).The use of plant extracts was reported to inhibit BYMV by 99% and develop induced resistance against the virus (Mahdy et al., 2007).Clove oil was applied against several microorganisms including plant viruses dueto anti-viral activity (Bhowmik et el., 2012; Iftikhar et al., 2013).The possible anti-microbial action for clove oil is attributed to eugenol which consists about 85-92% of total clove oil content (Bhowmik et el., 2012). Eugenol was found to enhance systemic resistance against *Tomato yellow leaf curl virus*(TYLCV) infection (Sun et al., 2016). Antimicrobial activity of bitter almond oil was reported againsthuman pathogenic bacteria(Gomaa , 2013). However, based on our knowledge antiviral activity of bitter almond oil against plant viruseshas not been tested yet. Recent research demonstrated that essential oils represent promising pesticide alternatives to control plant diseases (Koul et al., 2008). This study aimed at assessment the antiviral activity of clove and bitter almond oils against BYMV.

MATERIALS AND METHODS

BYMV infected seeds from a local variety were grown in greenhouse at college of Agriculture-University of Baghdad. Clove oil and bitter almond oil commercially available were used. Emerged seedlings were tested for BYMV infection using BYMVIDENTIKIT ELISA kit (Neogen Europe Ltd, UK) following the manufacturer`s instructions. BYMV positive symptomatic faba bean plants were sprayed twice with 5% clove oil and bitter almond oil (7 replicates each) individually, with two weeks interval. Sprayed plants were washed with commercial washing liquid and sterile distilled water two days after eachtreatments. To assess antiviral activity for clove and bitter almond oils, treated plants

were tested for BYMV infection 30 days after last treatments, using ELISA. BYMV concentration was estimated based on absorbent values at 405 nm using ELISA reader (BioTek, USA). The effect of clove and bitter almond oils on treated plant was assessed by measuring plant growth parameters. The following were measured (Plant length, Number of flowers, number of fruits and fruit weight).

RESULTS AND DISCUSSION

Results revealed clove and bitter almond oils reduced symptoms significantly when no symptoms were shown on infected faba bean plants compared to untreated infected control. ELISA technique confirmed that clove oil (CO) antiviral activity was higherthan bitter almond oil (BAO) when the average absorbent values at OD 405 nm were0.72 and 1.33respectively compared to 2.92and 0.34 for untreated infected (UIC) and negative (UNC) controlsrespectively (Figure 1).Except for number of flowers, BYMV infected faba bean plants treated with BAO showed better plant growth parameters than those treated with COcompared to UIC and UNC controls (Figure 1). The average of plant heights treated withBAO and CO were 30.2 and 27.4cm, respectively,Whereas they were24.7 and 45.4 cm forUIC and UNCcontrols respectively.The total number of flowers was slightly low inBAO treated plantswith 48 flowerscompared to CO,UNC and even UIC with 53, 84 and 50 flowers, respectively.

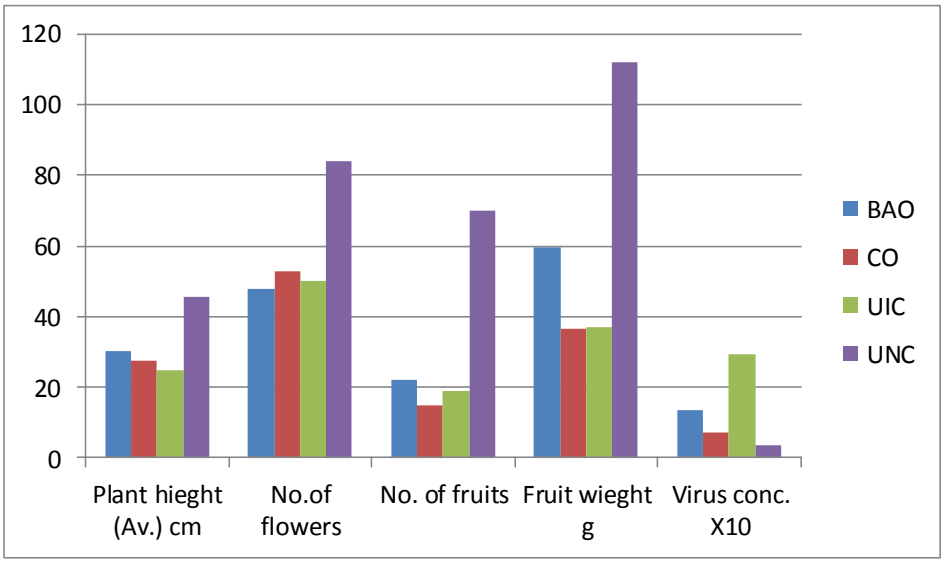

Figure 1: Comparisons of the effect of clove oil (CO) and bitter almond oil (BAO) on plant growth parameters compared to untreated infected (UIC) and negative (UNC) controls. The diagram shows virus concentrations for each treatments.

However BAO treated plant showed higher fruit numbers and weight which were up to 22 pods and 59.5 g, respectively, compared to CO treated plants with 15 pods and 36.5 g. Whereas, the total pod numbers and weights were 19 and 37 pods, 70 and 120 g for UNC and UIC, respectively. Based on symptomology and ELISA results, both CO and BAO showed antiviral activity when successfully reduced BYMV concentration in infected faba bean plants. Conceivably, they inhabited BYMV coat protein synthesis as essential oils have shown to inhibit mRNA expression of capsid protein gene (Iftikhar et al., 2013; Lu et al., 2013). CO content of eugenol, may explain the high antiviral activity, compared to BAO through inducing systemic resistance against BYMV (Sun et al., 2016). It has been demonstrated that eugenol could enhance systemic resistance against *Tomato yellow leaf curl virus*, through stimulating the production of endogenous nitric oxide (NO) and salicylic acid (SA) in tomato plants (Sun et al., 2016). However, CO treated plants showed a low pods quality compared to BAO or even untreated infected plants. It has been shown that eugenol has phytotoxic activity against plant growth parameters(Ajayi et al., 2014). The current results showed that commercial clove and bitter almond oils could be applied to control BYMV infecting faba bean. Essintial oils represent promising green pesticides, less toxic and antiviral compounds against plant virus diseases. However,some limitations, likephytotoxicity, availability and cost effectiveness should be addressed before applying essential oils to control plant viruses in Iraq. Our study presents the first application of bitter almond oil against BYMV and potential antiviral activity against plant viruses.

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