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ORIGINAL PAPER

Ovicidal, larvicidal and anti-ovipositional activities of *Bifora radians* and other plant extracts on the grape berry moth *Paralobesia viteana* (Clemens)

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Abstract Methanol extracts of *Bifora radians*, *Arctium lappa*, *Humulus lupulus* and *Xanthium strumarium* were tested against the North American grape berry moth, *Paralobesia viteana* in laboratory and greenhouse assays. Egg hatch was reduced by *B. radians* and *X. strumarium* extracts, whereas larval mortality was observed in response to *B. radians*, *X. strumarium* and *A. lappa.* Given the high mortality against egg and larval stages of this pest caused by extracts of *B. radians*, further studies were performed to determine the dose–response relationships between extracts of this plant and oviposition and egg hatch of *P. viteana*. There was a significant reduction in egglaying and egg hatch with increasing extract concentration, with 0.1% extracts providing 80% mortality and 1% extracts causing more than 90% control. This study demonstrates the potential of this plant extract for crop protection against a key pest of grapes and suggests that additional crop pests and field tests should be pursued to determine the efficacy of *B. radians* extracts as a biopesticide.

Keywords Plant extract · Grape · *Bifora radians* · *Arctium lappa* · *Humulus lupulus* · *Xanthium strumarium* · *Paralobesia viteana*

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Introduction

There is increasing interest in the use of plant-derived products for insect control in agricultural systems of the developed and developing worlds (Hall and Menn 1999; Isman 2006). The reasons for this growing attention include relative ease of access to bioactive compounds, greater access to organic markets and potentially higher safety to non-target organisms. In rural areas where access to technology is limited and supply systems can restrict availability of advanced pest management technologies, the ability to extract locally grown plants to make pest-active products has obvious benefits to small farmers. Plant-derived products can also be highly effective insecticides that in some cases are amenable to widespread cultivation in support of producing refined end-products for mass distribution (Isman 1999). Good examples of this latter approach can be seen in the plantings of *Azadirachta indica* for neem oil production and in *Chrysanthemum cinerariaefolium* that yields pyrethrum insecticides (Isman 2006).

Our research has explored the potential for native Turkish plants to provide locally available biopesticides for farmers in that region of Eurasia to protect crops from pests. Four plant species are of particular interest: Wild bishop (*Bifora radians* Bieb: Apiaceae), burdock (*Arctium lappa* L.: Asteraceae), hops (*Humulus lupulus* L.: Cannabaceae) and rough cocklebur (*Xanthium strumarium* L.: Asteraceae). These plant species are known to produce many secondary chemicals, e.g., phenolics, terpenoids and alkaloids (Latrasse et al. 1991; Stevens et al. 1997; Ma et al. 1998; Bruneton 1999; Taylor et al. 2003;) and have been tested against various key insect pests, including Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera Chrysomelidae), *Thaumetopoea solitaria* Frey.

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(Lepidoptera: Thaumetopoeidae), grape berry moth, *Paralobesia viteana* Clemens (Lepidoptera: Tortricidae), obliquebanded leafroller, *Choristoneura rosaceana* Harris (Lepidoptera: Tortricidae) and redbanded leafroller, *Argyrotaenia velutinana* Walker (Lepidoptera: Tortricidae) (Gökçe et al. 2005, 2006, 2007a, b, 2010; Er et al. 2009). Recent research by Gökçe et al. (2007a) has demonstrated the biological activity of methanol extracts of these plants against the Colorado potato beetle with protection from feeding injury and mortality of beetles in laboratory bioassays. A follow-up study examined the dose–response relationships of extracts of *H. lupulus* against *L. decemlineata*, finding that behavioral effects were more pronounced in larval life-stages and also that the responses were not always linear (Gökçe et al. 2011).

In addition to contact and ingestion toxicities to Colorado potato beetle, the antioviposition, antifeedant and repellent activities of *H. lupulus* that has been demonstrated against *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae) (Gökçe et al. 2007b) and previously against *Tetranychus urticae* Koch (Arachnida: Tetranychidae) (Jones et al. 1996, 2003) suggest that individual plant extracts may be broadly active against plant- feeding pests in multiple pest guilds. To broaden the scope of testing of the same plant extracts, we initiated studies with grape berry moth (GBM). This insect is a primary insect pest of grapes in eastern North America, causing direct damage from larval feeding inside berries and indirect damage through opening fruit to infestation by opportunistic larvae (Dennehy et al. 1990; Isaacs et al. 2011). A first series of studies on behavioral effects and peripheral sensitivity of extract odors to *P. viteana* insect was reported previously (Gökçe et al. 2006). The current study addresses the plant protection potential of these plant extracts by exploring their effects on survival of egg, larval and adult *P. viteana*, and egglaying by this species.

Materials and methods

Insect culture

Moths for these bioassays were selected from a colony of *P. viteana* maintained at Michigan State University, reared using methods modified from Nagarkatti et al. (2001). Red grapes used in the colony were purchased from a local store, surface sterilized by immersion in a 1% bleach solution for 5 min, rinsed thoroughly in deionized water, dried and presented to *P. viteana* moths. Three times each week, new clusters were added to the colony and infested grapes were removed and stored in plastic containers at room temperature (23 ± 3°C). Under these conditions, eggs eclosed in approximately 4–6 days yielding a steady supply of neonates that were transferred to a meridic diet (Nagarkatti et al. 2000) containing blended grapes.

Plant material

The detailed experimental protocol for preparing plant extracts was described by Gökçe et al. (2005). *B. radians* and *A. lappa* samples were collected during flowering stage as mature fruits for *X. strumarium* and mature flower cones for *H. lupulus* were picked up the summer and fall of 2006–2008 in Taşlıçiftlik, Tokat, Turkey. Samples were dried at room temperature for 3 weeks in the dark and subsequently were ground to fine size with a mill (M 20 IKA Universal Mill, IKA Group). Ground plant material was stored in 2,000-ml glass jars at 18 ± 2°C in the dark. Fifty grams of a plant sample was placed into 1,000-ml Erlenmeyer flasks with 500 ml of methanol (Sigma-Aldrich). The flasks were covered with aluminum foil, placed on a horizontal shaker (HS 260 Basic, IKA Group) and shaken (120 oscillations/min for 24 h) in the dark. The suspension was filtered through two layers of cheese cloth and transferred into a 250-ml evaporating flask, and excess solvent was evaporated in a rotary evaporator (RV 05 Basic 1B, IKA Group) at 32 ± 2°C. The resulting residue was weighed and eluted with HPLC grade acetone (Sigma–Aldrich) to yield a 20% (w/w) plant suspension.

Ingestion toxicity of plant extracts to GBM larvae

The ingestion toxicity of plant extracts to *P. viteana* larvae was tested on the meridic diet treated with plant extracts in a no choice context. One hundred milligrams of each plant extract in 100 µl of HPLC grade acetone was incorporated per g of freshly prepared meridic diet, to give 10% (w/w) concentrations. Each plant extract diet treatment was formulated separately. For the control treatment, 100 µl of HPLC grade acetone was added per gram of the diet. Three grams of each diet treatment was transferred into 240-ml soufflé cups and allowed to solidify. Thereafter, three neonate larvae were transferred into each cup. The larvae were incubated at 25 ± 2°C and in a 16:8 (L:D) light regime for 10 days. Larvae were checked daily for mortality. The experiment was repeated on three different days (blocks), and 15 larvae were exposed to each plant extract or control treatment per block. The initial screening bioassay data were corrected for mortality in the controls using Abbott's formula (Abbott 1925) and then normalized using an arcsine transformation ($p' = \text{arcsine}\sqrt{p}$) (Zar 1999). Transformed data were submitted to a randomized complete block analysis of variance (ANOVA) ($\alpha < 0.05$), and differences between treatments were compared using Tukey's test ($\alpha < 0.05$) (Minitab Release 14, McKenzie and Goldman 2005).

Dose–response bioassay with *B. radians* against GBM larvae

Additional toxicity studies were conducted using the *B. radians* extract based on its substantial toxic effects on *P. viteana* larvae in the initial assay described above. Five concentrations of the extract, 0.001, 0.01, 0.1, 1 and 10% (w/w), were prepared as described above. For the control treatment, 100 µl of HPLC grade acetone was added per gram of the diet. Three grams of each diet treatment was transferred into 240-ml soufflé cups and allowed to solidify. Ten neonate larvae were transferred into each cup and incubated at 25 ± 2°C and in a 16:8 (L:D) light regime for 10 d. Larvae were checked daily for mortality. There were three replicates in each treatment, and the whole experiment was repeated three times. GBM larval mortality data obtained from the dose–response bioassay utilizing *B. radians* were analyzed by probit analysis using the PoloPlus program (Robertson et al. 2002) to calculate lethal concentration 50 and 90 values (LC50 and LC90) and the regression line slope.

Ovicidal activity screening

The ovicidal activity of plant extracts was tested on grape berries carrying eggs of *P. viteana*. Grape clusters containing 5–10 fully grown berries were covered with a mesh bag (200 mm in width and 200 mm in length), and ten 1- to 3-day-old *P. viteana* moths adult (five female: five male) were transferred to each bag and allowed to lay eggs for 24 h. After that time, GBM adults were removed from the bag and 2–3 berries with 10–20 eggs laid on them were used for the experiment.

Berries were subsequently treated by dipping them individually into 100 ml plant extract suspensions (10% w/w) for 10 s using forceps and drying them in a fume hood for 20 min on 9-cm-diameter Petri dishes loaded with sterile Whatman filter paper. In the control treatment, berries were dipped into HPLC grade acetone for 10 s and transferred into Petri dishes. After drying each cluster, berries with eggs were hung by the stem using a cotton string attached to the lid inside a 16-oz plastic cup (80 mm in height and 110 mm in diameter). The eggs were incubated in these containers (one berry per container) at 25 ± 2°C and on a 16:8 (L:D) photocycle for 10 days. The numbers of hatched and unhatched eggs were counted on each berry. A randomized block design was used in this study, with each block consisting of four treatments and one control. The whole experiment was replicated seven times. The ovicidal activity screening experiment data were calculated as percentages [Number of hatched eggs/(Number of hatched eggs + number of unhatched eggs)]. The data were subjected to arcsine transformation (Zar 1999) to normalize the error residuals and fulfill assumptions of ANOVA. Transformed data were analyzed with analysis of variance (ANOVA) ($\alpha < 0.05$), and differences between treatments were compared using Tukey's test ($\alpha < 0.05$) (Minitab Release 14).

Dose–response against GBM eggs

Additional toxicity studies against GBM eggs were conducted using *B. radians* and *X. strumarium* extracts based on their substantial toxic effects on the eggs in the initial screening assay. Grape berries were infested with *P. viteana* eggs as described above. Grape berries were treated with *B. radians* and *X. strumarium* extracts at 0.001, 0.01, 0.1, 1 and 10% (w/w), by dipping them individually into 100 ml plant extract suspension for 10 s as described above. In the control treatment, berries were treated with acetone for 10 s. Grape berries with eggs were incubated at 25 ± 2°C and on a 16:8 (L:D) light regime for 10 days. The numbers of hatched and unhatched eggs were counted on each berry. There were three replicates for each concentration of each plant extract and for the control treatment in each trial. The whole trial was repeated three different times. Dose response data obtained from testing various concentrations of *B. radians* extract on GBM eggs were analyzed by probit analysis using the PoloPlus program to calculate lethal concentration 50 and 90 values (LC50 and LC90) and the regression line slope. Differences between LC50 and LC90 values were tested using an LC50 and LC90 ratio test (Robertson et al. 2007).

Oviposition assays

The effect of *B. radians* extract on oviposition by female *P. viteana* was tested in greenhouse conditions. Three-year-old grape vines, *Vitis labrusca*, cv. Concord, each with 6–8 grape clusters were used in this experiment. Extracts with 0.001, 0.01, 0.1, 1 and 10% (w/w) concentrations were prepared in acetone from the stock suspension as described above. Grape clusters were sprayed with one of these solutions or the control to drip using a handheld sprayer (Lansing Sanitary Supply Inc., MI) and then dried at room temperature. After drying, grape clusters were covered with curtain mesh bags. Ten 1- to 3-day-old grape berry moth adults (five female: five male) were transferred to each bag and allowed to lay eggs for 5 days. A 5% sucrose solution in plastic cups with dental cotton wick protruding from their lids was provided to aid moth survival. After 7 days, the number of individual eggs was counted on each berry cluster. A randomized block design was used in this study, with each block consisting of five concentrations of *B. radians* and a control. The whole experiment was replicated 4 times.

In the oviposition test, egg numbers laid per female were submitted to analysis of variance (ANOVA) ($\alpha < 0.05$), and differences between treatments were compared using Tukey's test ($\alpha < 0.05$) (Minitab Release 14).

Results

The tests of ingestion toxicities of the four plant extracts showed that *B. radians*, *A. lappa* and *X. strumarium* were toxic to GBM larvae (Fig. 1). There were significantly greater levels ($F = 49.6$; $df = 4,10$; $P < 0.001$) of mortality of larvae treated with plant extracts compared with the control, except for *H. lupulus* ($P < 0.05$). *B. radians* induced the highest mortality, causing all tested larvae to die, whereas *H. lupulus* extract produced only 13% mortality.

There were significant ($F = 57.86$; $df = 4,29$; $P < 0.001$) effects of the plant extracts on viability of GBM eggs (Fig. 2). Treatments of eggs with *B. radians* and *X. strumarium* significantly reduced the proportion of eggs that hatched. The hatched rates for these two extracts were 8.19 and 8.32%, respectively. The larvae coming out of treated eggs with these plant extracts did not survive and they died 1–3 days after hatching (data not presented). *H. lupulus* and *A. lappa* did not significantly reduce egg hatch ($P > 0.05$). Larvae emerged from the eggs treated with these extracts and passed to the next stadium with low observed mortality.

The results of dose–response relationship for grape berry moth eggs and larvae treated with *B. radians* and *X. strumarium* extracts are presented in Table 1. Although the application method of the extract was different, the LC50 values for *B. radians* varied, depending on the tested

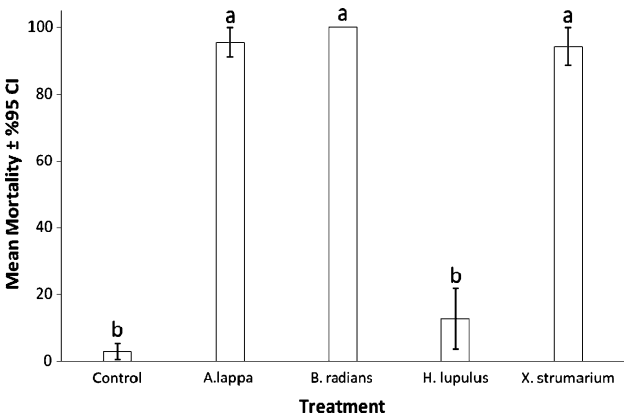

Fig. 1 Mean mortality (±95% CI)* of grape berry moth larvae (*Paralobesia viteana*) to four plant extracts. *Different lower case letters* represent statistically significant differences between treatments according to Tukey's multiple comparison test of arcsine transformed data ($P < 0.05$). *Data were arcsine transformed for analysis. Back-transformed data are presented

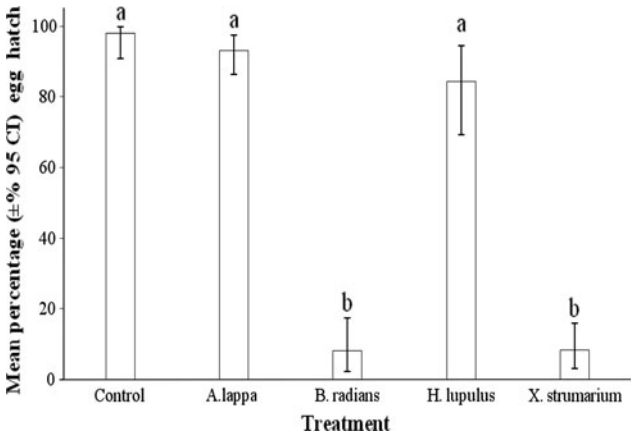

Fig. 2 Ovicidal activity (±95% CI) of four plant extracts to grape berry moth (*Paralobesia viteana)* eggs. *Different lower case letters* represent statistically significant differences between treatments according to Tukey's multiple comparison test of arcsine transformed data ($P < 0.05$). *Data were arcsine transformed for analysis. Back-transformed data are presented

stage of GBM. The egg stage had the lowest LC_{50} value and appeared to be more sensitive to this extract than the larvae. While the egg stage had the narrowest fiducial limits for this extract, the fiducial limits for larvae were the widest among tested stages (Table 1).

The toxicity of plant extracts to the egg of GBM varied depending on the plant extracts. *B. radians* produced the lowest LC50 value, and it was nearly one-sixth of the LC value for *X. strumarium.* However, *X. strumarium* extract had steeper slope comparing with *B. radians* slope that indicates that the former extract toxicity to the GBM egg was more homogenous.

Comparison of the LC10, LC50 and LC90 values of *B. radians* and *X. strumarium* with a ratio test showed that there was a difference between these extracts for LC10 and LC50 (LC10 ratio 6.43; lower limit 1.43; upper limit 28.97 and LC50 ratio 4.47; lower limit 2.19 and upper limit 9.11). However, the LC90 values of *B. radians* and *X. strumarium* were not different as the ratio was 3.11 and lower and upper limits were 0.71 and 13.70, respectively.

The oviposition behavior of *P. viteana* was significantly affected by exposure to *B. radians* extracts ($F = 14.98$; $df = 5.12$; $P < 0.001$) (Fig. 3). All tested concentrations of *B. radians* caused some reductions in the number of eggs deposited, although reductions were significant only at 0.1% and above. Among the tested concentrations, the 10% (w/w) concentration induced the greatest antioviposition effect, with no eggs laid at this concentration. The total number of eggs laid by females on *B. radians* at the 0.1 and 1% concentrations were 7.33 and 3 eggs/per female, respectively, and there was no significant difference between these three concentrations (10, 1 and 0.1% w/w). Although the number of eggs found on control grape

Table 1 Dose–mortality responses of egg and larval stages of *Paralobesia viteana* treated with *Bifora radians* and *Xanthium strumarium* extracts

Treatment	Tested stage of *P. viteana*	Number of insect tested	Slope ± SE	LC_{50} (95% Confidence intervals)	LC_{90} (95% Confidence intervals)	χ^2
B. radinas	Egg	525	0.89 ± 0.14	2.46 (1.44–4.37)[a]	66.46 (25.99–346.74)[a]	12.90
X. strumarium	Egg	581	1.01 ± 0.14	11.02 (6.66–17.39)[a]	206.68 (114.29–500.49)[a]	13.21
B. radinas	Larvae	540	1.16 ± 0.16	10.97 (6.59–17.19)[b]	139.46 (78.29–352.57)[b]	8.74

[a] LC_{50} and LC_{90} values are plant extract in solvent (w/w) base

[b] LC_{50} and LC_{90} values are μg plant extract in per gram of diet

clusters compared with grapes treated with *B. radians* concentrations at 0.001 and 0.01% was not significantly different from the control, these two treatments also reduced the number of eggs laid from 24.33 eggs/per female (the control) to 18.33 eggs/per female and 16.00 eggs/per female, respectively.

Discussion

Development of new biopesticides based on plant extracts requires a thorough understanding of their potential activity against pests that growers need to protect their crops from. In this study, we used a key grape pest as a model system to compare different plant extracts. Among the tested plant extracts, *A. lappa*, *B. radians* and *X. strumarium* showed some potential as biopesticides for Lepidopteran pest control. However, *B. radians* appeared to have more chance for developing as a biopesticide with high ingestion and ovicidal toxicities and antioviposition activity.

Grape berry moth causes significant economic losses in vineyards across eastern North America and is currently managed using a range of conventional and new reduced risk insecticides (Jenkins and Isaacs 2007). However, there is interest in development and adoption of novel approaches that will provide benefits for resistance management and for growers interested in organic markets. Based on the results presented here, there is potential for use of *B. radians* extract to prevent cluster infestation by *P. viteana*, and further field trials are warranted. The bioassay results are encouraging that this extract can prevent oviposition through a repellency activity, particularly because moths did not lay any eggs on the highest concentration extract when caged more than 7 days, indicating that there was no habituation of females to the extract.

This study showed that two of the plant extracts evaluated (*B. radians* and *X. strumarium*) exhibit both ingestion toxicity and ovicidal activity to GBM. *A. lappa*, *B. radians and X. strumarium* produced high ingestion toxicity to GBM larvae. These results are in agreement with previous studies showing pronounced ingestion toxicity of *B. radians* and *X. strumarium* to obliquebanded leafroller larvae (Gökçe et al. 2010). The ingestion toxicity and ovicidal activity induced by the tested plant extracts, particularly *B. radians* and *X. strumarium*, suggest the potential for their future use against GBM as botanical insecticides. Further testing of biological activity with purified compound on GBM and also on other related pest species such

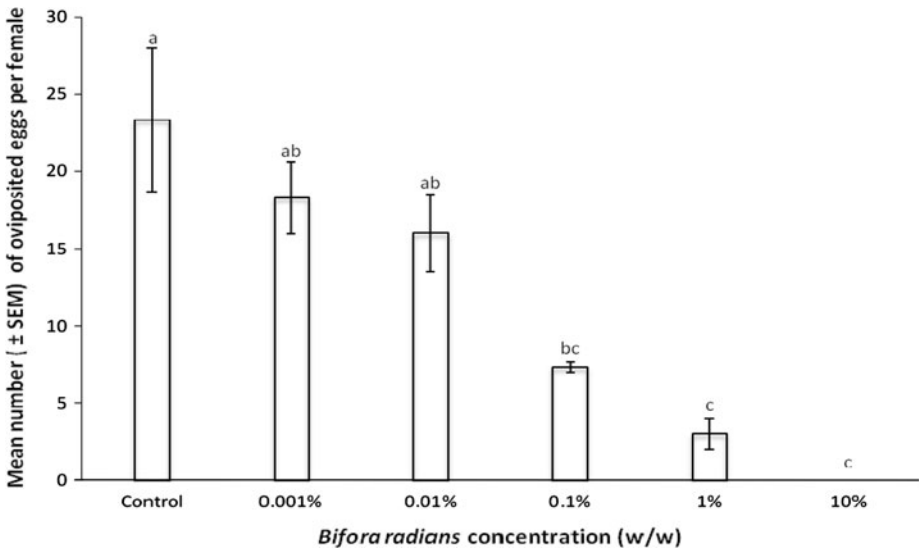

Fig. 3 Antiovipositional effect of different concentrations of *Bifora radians* to grape berry moth (*Paralobesia viteana)* females. *Bars* with the same letter are not significantly different ($P < 0.05$)

as European grapevine moth, *Lobesia botrana*, is required to explore the full potential of these extracts in pest management strategies.

In addition to contact and ingestion toxicities, secondary plant compounds have been tested against important pest species as antifeedants, repellents, insect growth regulators and antioviposition (Isman 1993; Gökçe et al. 2005, 2006). In some instances, the bioactivity of crude plant extracts on insects is comprised of both toxic and behavioral effects (Wheeler et al. 2001; Leatemia and Isman 2004; Akhtar and Isman 2004; Gökçe et al. 2006, 2010). Azadirachtin is a good example in that it has both toxic and behavioral effects and has been one of the most widely tested and successfully implemented botanical insecticides over the past two decades (Schmutterer 1995). In the present study, *B. radians* produced antioviposition activity on GBM females in addition to ingestion toxicity and ovicidal activity, while *X. strumarium* exhibited toxic effects when ingested. *B. radians* are also known to deter oviposition of obliquebanded leafroller females (Gökçe et al. 2005). Our results suggest that potential future application of these extracts or their active components for GBM control may exploit more than one mode of action. Although during 1 week of incubation, the female did not lay any eggs at the higher concentration of *B. radians*, and further experiments are needed to determine whether prolonged exposure of GBM females to *B. radians* extract decreases the antiovipostion effects over time.

Bifora radians has a distribution in the eastern Mediterranean and the Caucuses region (Davis, 1972). It is a thermophilic plant that adapts well to nitrogen-rich and alkaline soils. Adaptation of the plant to cultivation has not been pursued, but the plant grows well as a weed of some spring crops and would be adaptable to cultivation. *B. radians* contains many terpenoids mainly (E)-2-tridecenal and (E)-2-tetradecenal and also alkaloids (Baser et al. 1998; A. Gokce unpublished work), and the essential oil has been characterized by Latrasse et al. (2005). *B. radians* methanol extract repels many important pest species belonging to Lepidoptera and Coleoptera (Gökçe et al. 2005, 2006, 2007b, 2010). Future studies will need to be conducted to determine whether the biological activity of *B. radians* against GBM larvae and adults is mediated by terpenoid or alkaloid components. Furthermore, identification of the bioactive components of *B. radians* may allow development of botanical insecticides with greater potency than the crude plant extract evaluated here.

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