

四种非寄主植物精油对小菜蛾的生物活性

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摘要: 为寻找小菜蛾 *Plutella xylostella* 绿色防控方法,于室内测定辣蓼、迷迭香、芸香和紫苏4种非寄主植物精油对小菜蛾的生物活性。结果表明,辣蓼植物精油对小菜蛾2龄幼虫的毒杀作用最强,24、48和72 h时的致死中浓度LC₅₀分别为2.18、1.78和1.46 μg/μL,芸香植物精油次之;当芸香植物精油浓度为15 μg/μL时,小菜蛾3龄幼虫的选择拒食率最高,作用24 h和48 h时分别为100.00%和92.84%,而在10 μg/μL浓度下作用24 h和48 h时非选择拒食率达到最高值,分别为65.72%和42.72%;在4种植物精油中,芸香植物精油对小菜蛾3龄幼虫的生长抑制作用最明显,相对生长率最低,为-34.72%,生长抑制率最高,为133.74%,辣蓼植物精油的生长抑制效果次之,迷迭香和紫苏植物精油的抑制作用相对较差;小菜蛾雌、雄成虫对4种植物精油均表现出一定的触角电位反应和驱避活性,其中,芸香植物精油的驱避效果最明显;气相色谱-质谱分析结果显示,芸香植物精油的主要成分为β-石竹烯、(+)-α-蒎烯和β-蒎烯。表明芸香植物精油对小菜蛾具有显著的生物活性,具备开发为小菜蛾靶标杀虫剂的潜力。

关键词: 小菜蛾; 芸香植物精油; 生物活性

Bioactivities of the essential oils derived from four species of non-host plants against diamondback moth *Plutella xylostella*

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Abstract: In search for the green prevention and control method of diamondback moth *Plutella xylostella*, the biological activities of the essential oils from four non-host plants, *Polygonum hydropiper*, *Rosmarinus officinalis*, *Ruta graveolens* and *Perilla frutescens*, against *P. xylostella* were determined in the laboratory. The results showed that the toxicity of *P. hydropiper* plant essential oil against the 2nd-instar larvae was the strongest, with a median lethal concentration (LC₅₀) value of 2.18, 1.78 and 1.46 μg/μL at 24, 48 and 72 h, respectively, followed by *Ru. graveolens* plant essential oil. The choice antifeedant rate of *Ru. graveolens* plant essential oil at 15 μg/μL against the 3rd-instar larvae was the highest, which was 100.00% and 92.84% at 24 h and 48 h, respectively. Its non-choice antifeedant rate reached the peak values at the concentration of 10 μg/μL, which was 65.72% and 42.72% at 24 h and 48 h. *Ru. graveolens* plant essential oil had the most obvious inhibitory effect on the growth of the 3rd-instar larvae among the four species of plant essential oils, resulting in a lowest relative growth rate of -34.72% and a highest growth inhibition rate of 133.74%, followed by *P. hydropiper* plant essential oil, and *Ro. officinalis* and *P. frutescens* plant essential oils exhibited a relatively poor inhibitory effect. The female and male adults of *P. xylostella* displayed certain electroantennogram (EAG) responses and repellent activi-

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ties to the four plant essential oils. *Ru. graveolens* plant essential oil showed the most obvious repellent effect. The result of gas chromatography-mass spectrometer (GC-MS) assay showed that the main components of *Ru. graveolens* plant essential oil were β -caryophyllene, (+)- α -pinene and β -pinene. It indicated that *Ru. graveolens* plant essential oil had significant biological activity against *P. xylostella*, and had the potential to be developed as an insecticide for *P. xylostella*.

Key words: *Plutella xylostella*; *Ruta graveolens* plant essential oil; bioactivity

小菜蛾 *Plutella xylostella* 属鳞翅目菜蛾科, 寡食性, 是一种生命周期短、繁殖系数高、世代重叠严重和适应能力强的世界性害虫, 对十字花科蔬菜的产量、品质和生长造成毁灭性为害, 全世界每年因小菜蛾造成的经济损失和引发的防治费用高达 40 亿~50 亿美元(Furlong et al., 2013)。尽管自然界存在几种小菜蛾的寄生性天敌, 然而它们的寄生受多种因素限制, 以至于无法满足农业需求。目前关于小菜蛾的防治主要采用化学合成农药, 而过度依赖这种单一的防控手段也引发了许多新问题(Liang et al., 2003; Boulogne et al., 2018), 如害虫的抗药性逐年增强, 对天敌群体造成严重破坏, 食品中农药残留较高等(Wang et al., 2015; Benelli et al., 2019; Falkowski et al., 2020)。小菜蛾是首个对杀虫剂双对氯苯基三氯乙烷(dichlorodiphenyltrichloroethane, DDT)(Ankersmit, 1953) 和苏云金芽孢杆菌 *Bacillus thuringiensis* (Bt)(Baxter et al., 2011) 毒素产生抗性的害虫, 也是少数几种几乎对所有主要杀虫剂均产生抗性的昆虫之一(Heckel et al., 1999; Tabashnik et al., 2011)。此外, Fang et al.(2017)研究表明化学农药仅有 0.3% 左右能进入目标生物体, 其余大部分农药最终都会流入环境, 对环境安全造成严重危害。因此, 小菜蛾的综合治理仍面临着巨大的压力, 寻找节本增效、优质安全、绿色发展的防控体系具有重大意义(吴孔明, 2018; Dougoud et al., 2019; Iqbal & Pavela, 2019)。

植物在长期的协同进化过程中产生大量与昆虫进行复杂信息“交流”的次生代谢产物(钦俊德, 1987)。这些次生物属于挥发性物质, 广泛存在于植物精油中, 其中多种成分对昆虫有杀虫(Murcia-Meseguer et al., 2018; Vatandoost et al., 2018; Soonwera & Cotchakaew, 2019)、拒食(Akhtar et al., 2015; Azeem et al., 2020) 和驱避(Gao et al., 2019; Satyal et al., 2019; Yang et al., 2019) 等生物活性。近年来, 在害虫防治中, 植物精油受到越来越多的关注(Singh et al., 2011; Akhtar et al., 2012)。更为重要的是, 一些研究发现多种精油及其成分比传统的合成

杀虫剂更有效(Liu et al., 2012; Zhao et al., 2012; Liang et al., 2013)。因此, 从安全性、抗药性、特异性以及环境等多因素考虑, 来源于芳香植物的天然植物精油已成为害虫防治的重要方法之一(Benelli, 2016a, b; Azeem et al., 2019)。辣蓼 *Polygonum hydropiper*(Ayaz et al., 2016a)、迷迭香 *Rosmarinus officinalis*(Rahbardar & Hosseinzadeh, 2020)、芸香 *Ruta graveolens*(Coimbra et al., 2020) 和紫苏 *Perilla frutescens*(Igarashi & Miyazaki, 2013) 是我国传统的中草药材, 具有精油含量高、原材料丰富、易于种植和养护成本低等优点, 已被广泛用于医药领域。此外, 关于其在杀虫方面的研究更是屡见不鲜, 如 Kundu et al.(1970) 测试了辣蓼粗提物对赤拟谷盗 *Polygonum hydropiper* 的毒杀效果, 发现其乙醇粗提物处理成虫比氯仿粗提物的毒力更强; Ayaz et al., (2014) 发现辣蓼粗提物对蚯蚓 *Pheretima posthuma* 和蛔虫 *Ascaridia galli* 成虫具有驱虫作用; Zhang et al.(2015) 发现迷迭香能显著调控茶尺蠖 *Ectropis obliqua* 的行为; Perera & Karunaratne(2016) 发现芸香植物精油对米象 *Sitophilus oryzae* 有很强的杀虫和驱避作用; Ghabbari et al.(2018) 发现芸香乙醇提取物对地中海实蝇 *Ceratitis capitata* 有显著的杀虫作用, 作用 24 h 后半数致死量 LD₅₀ 和 90% 致死量 LD₉₀ 分别为 3.64 μL 和 6.01 μL; You et al.(2014) 发现紫苏植物精油对烟草甲 *Lasioderma serricorne* 有毒性和驱避性。

为寻找对小菜蛾室内防治效果较好的精油, 首先分别采用浸叶法、叶碟法和饲喂称重法测试辣蓼、迷迭香、芸香和紫苏 4 种植物精油对小菜蛾幼虫的毒杀、拒食和生长抑制活性; 利用触角电位仪(electroantennogram, EAG) 和 Y 型嗅觉仪测定小菜蛾成虫对这 4 种植物精油的电生理和行为反应; 通过气相色谱质谱联用仪(gas chromatography-mass spectrometer, GC-MS) 对生物活性最好的植物精油成分进行分析鉴定, 以期为小菜蛾生态可持续控制措施提供新的思路与方法, 为开发新型、可替代的病虫害防治管理策略提供理论依据。

1 材料与方法

1.1 材料

供试虫源和植物:小菜蛾蛹和幼虫自山西农业大学试验站蔬菜地中收集,于植物保护学院昆虫神经行为与感觉生物学实验室中连续饲养多代,饲养温度为(25±1)℃、相对湿度为(70±5)%、光周期14 L:10 D,幼虫取食萝卜苗 *Raphanus sativus*,成虫以10%蜂蜜水补充营养,选取2龄和3龄新生幼虫以及2日龄成虫供试。在山西农业大学校内温室中用蛭石培育萝卜苗,未施用农药,品种为春秋九斤王,待长至株高5 cm左右供幼虫取食;甘蓝 *Brassica oleracea* 于校内温室中种植,未施用农药,品种为中甘11号,待成熟后,选择新鲜且大小均匀的甘蓝,取其外层较平整且厚薄一致的叶片制得直径分别为5 cm和1.5 cm的叶盘供试。

供试植物精油及试剂:辣蓼、迷迭香、芸香和紫苏4种植物精油,吉安市中香天然植物有限公司;石蜡油,美国Sigma-Aldrich公司;其他试剂均为国产分析纯。

仪器:触角电位仪由IDAC-2信号采集控制器、MP-15微动操作仪、CS-55刺激气流控制器和Syntech分析软件组成,荷兰Syntech公司;嗅觉仪由QC-1S大气采样仪、活性炭过滤装置、空气加湿瓶、LZB-3WB空气流量计和Y型管组成,自制;QC-1S大气采样仪,北京市劳动保护科学研究所;LZB-3WB空气流量计,常州市科德热工仪表有限公司;Y型管,3个臂长均为18 cm,两侧臂的夹角为70°,管内径2.6 cm,建湖县明特玻璃仪器厂;气相色谱质谱联用仪,美国Thermo公司;Spectra 360导电胶,美国Parker公司。

1.2 方法

1.2.1 植物精油对小菜蛾幼虫毒杀活性的测定

采用浸叶法(Park et al., 2002)测定辣蓼、迷迭香、芸香和紫苏这4种植物精油对小菜蛾2龄幼虫的毒杀作用。用丙酮分别稀释这4种精油,将每种精油分别制备成6种浓度的测试液,即0.3125、0.625、1.25、2.5、5和10 μg/μL。将直径5 cm的甘蓝叶盘浸入测试液中10 s,室温下置于通风处蒸发,置于装有润湿滤纸的玻璃培养皿中,以防止干燥。将20头饥饿处理4 h的2龄幼虫转移到培养皿中,用封口膜密封后于实验室条件下饲养,每24 h观察虫体的死亡情况,连续观察72 h,用细毛刷轻轻触碰虫体,若无反应则认为死亡。以丙酮处理的叶盘作为对照,每

个处理重复3次。采用Probit回归计算致死中浓度LC₅₀和毒力回归方程。

1.2.2 植物精油对小菜蛾幼虫拒食活性的测定

采用叶碟法(田万里等,2018)测定小菜蛾3龄幼虫对4种植物精油的选择性拒食活性和非选择性拒食活性。用丙酮分别稀释这4种精油,每种精油分别制成5、7.5、10和15 μg/μL四种浓度的测试液。将直径1.5 cm的甘蓝叶盘分别浸入每种精油的4个浓度中,10 s后取出于室温下蒸发干燥,丙酮处理的对照叶盘和精油处理的试验叶盘各2片交叉排列放到内有湿润滤纸的一个培养皿中,取2头生长状态一致且饥饿处理4 h的小菜蛾3龄幼虫置于培养皿内,分别于24 h和48 h后取出叶盘,使用Photoshop 6软件计算取食面积(肖强等,2005),计算选择拒食率(Isman et al., 1990),选择拒食率=(对照组取食面积-试验组取食面积)/(对照组取食面积+试验组取食面积)×100%,每个处理重复6次;另取2片相同处理的叶盘和2头生长状态一致且饥饿处理4 h的小菜蛾3龄幼虫放入另一培养皿中,丙酮处理的叶盘作为对照。分别于24 h和48 h后取出叶盘,使用Photoshop 6软件计算取食面积(肖强等,2005),计算非选择拒食率(Huang et al., 2008),非选择拒食率=(对照组取食面积-试验组取食面积)/对照组的取食面积×100%,每个处理重复6次。

1.2.3 植物精油对小菜蛾幼虫生长抑制活性的测定

采用饲喂称重法(程丽坤等,2007)测定4种植物精油对小菜蛾3龄幼虫的生长抑制活性,精油浓度同1.2.2。小菜蛾3龄幼虫饥饿处理4 h后,每5头幼虫为1组,称取各组幼虫体重,保证每组幼虫体重在一定范围内。分别用每种植物精油的每个浓度对5 cm的甘蓝叶盘进行处理后喂食幼虫;以取食丙酮处理叶盘的幼虫为对照。每个浓度处理重复6次,分别于处理24 h和48 h后对每组幼虫再次称重,根据Huang et al., (2000)和田万里等(2018)公式计算相对生长率和生长抑制率,相对生长率=(试验后幼虫的体重-试验前幼虫的体重)/(试验前幼虫的体重×天数)×100%;生长抑制率=(对照组体重增加量-处理组体重增加量)/对照组体重增加量×100%。

1.2.4 小菜蛾成虫对植物精油EAG反应的测定

参照Wu et al.(2020)方法测定小菜蛾成虫对4种植物精油的EAG反应。每日18:00—22:00取羽化24 h、大小一致、触角完整且活跃的成虫,用手术刀片迅速将1根触角从基部切下,端部切掉少许以提高导电性;使用Spectra 360导电胶将触角固定在叉

形电极上，并接通电极。用石蜡油将4种植物精油浓度分别稀释为1、10、100和500 μg/μL，从低到高浓度依次进行测试，每种植物精油测试前和测试后分别以石蜡油为对照进行测试。取10 μL测试液滴于0.5 cm×3 cm的三角形滤纸条上，将其置于巴斯德管中，连接气体刺激控制装置，气流500 mL/min，待基线平稳后开始测试。每种植物精油每个浓度测试雌雄成虫触角各6根，每根触角每次持续测量0.5 s，共测量6次，每2次间隔60 s，以确保触角传感器功能完全恢复，用Syntech软件记录电位信号。计算时从测试液刺激引起的电位反应振幅中扣除石蜡油引起的反应值。

1.2.5 小菜蛾成虫对植物精油行为反应的测定

参照徐伟等(2018)方法利用Y型嗅觉仪测定小菜蛾成虫对4种植物精油的行为反应。试虫状态和测试浓度同1.2.4。以大气采样仪的出口作为气流源，嗅觉仪两臂的气流流速均设置为350 mL/min。取10 μL测试液滴到0.5 cm×3 cm的长方形滤纸条上，组成气味源，放入Y型管一个侧臂中，另一侧臂中放入含有石蜡油的滤纸条(对照)。测试时，于Y型管主臂管口释放单头成虫，待其爬至主臂1/2处开始计时，到达任一个侧臂9 cm处且停留30 s以上时，视为选择，并记录对味源或对照的选择情况；当放入5 min后无明显选择趋向视为无反应。每测试1头，Y型管两臂互调方向；每测试2头，更换干净的Y型管。每种植物精油每个浓度分别测试成功选择的雌雄成虫各30头。每次测试完毕，用丙酮清洗玻璃仪器和硅胶管，并于80℃烘箱中加热烘干2~3 h。

1.2.6 植物精油成分的GC-MS分析

采用GC-MS对小菜蛾生物活性最好的植物精

油成分进行分析，其中采用GC分离精油混合物的成分，采用MS识别分离出的成分。气相色谱仪装有30 m×0.25 mm×0.25 μm(柱长×内径×膜厚)的TG-5MS毛细管柱。气相柱箱于50℃下保持2 min，以4℃/min的速度升至120℃，保持2 min，以20℃/min的速度升至280℃，保持4 min；溶剂延迟3 min；以50:1的分流比进样1 μL；高纯氦气用作载气，流速为1.0 mL/min；进样口和离子源温度分别保持在250℃和280℃温度下；电离方式EI，电子能量70 eV，扫描范围为45~500质核比。

1.3 数据分析

使用SPSS 21.0软件对试验数据进行统计分析。采用单因素方差分析，同处理时间小菜蛾对同种植物精油不同浓度的选择拒食率、非选择拒食率、相对生长率和生长抑制率之间以及小菜蛾同性别成虫对同种植物精油不同浓度EAG值之间采用Tukey's HSD法进行差异显著性检验；采用卡方检验对小菜蛾雌雄成虫和对照选择行为进行差异显著性检验；其他均采用Student's t测验法进行差异显著性检验。

2 结果与分析

2.1 4种植物精油对小菜蛾幼虫的毒杀活性

小菜蛾2龄幼虫对4种植物精油均有一定的敏感性，但存在差异。随着精油作用时间的增加，毒杀作用不断增强。辣蓼植物精油对小菜蛾2龄幼虫的毒杀作用最强，处理24、48和72 h后LC₅₀分别为2.18、1.78和1.46 μg/μL。4种植物精油毒力从高到低依次为辣蓼、芸香、迷迭香和紫苏(表1)。

表1 4种非寄主植物精油对小菜蛾幼虫的毒杀作用

Table 1 Toxicities of four non-host plant essential oils against larvae of *Plutella xylostella*

供试精油 Essential oil tested	处理时间 Exposure time/h	回归方程 Regression equation	LC ₅₀ /(μg/μL)	95%置信区间 95% confidence interval/(μg/μL)	χ ²	P
辣蓼 <i>Polygonum hydropiper</i>	24	y=4.982x-1.681	2.18	1.68~2.65	22.21	0.001
	48	y=4.717x-1.178	1.78	1.35~2.18	19.59	0.003
	72	y=4.186x-0.686	1.46	1.02~1.84	18.78	0.005
迷迭香 <i>Rosmarinus officinalis</i>	24	y=3.488x-1.953	3.63	2.72~4.61	27.69	<0.001
	48	y=3.192x-1.404	2.75	1.87~3.59	29.57	<0.001
	72	y=2.349x-0.583	1.77	0.66~2.64	33.64	<0.001
芸香 <i>Ruta graveolens</i>	24	y=3.595x-1.623	2.83	2.13~3.49	22.05	0.001
	48	y=3.435x-1.113	2.11	1.48~2.67	21.84	0.001
	72	y=4.971x-2.907	1.98	1.42~2.49	19.40	0.004
紫苏 <i>Perilla frutescens</i>	24	y=4.917x-3.241	4.56	-	287.76	<0.001
	48	y=3.125x-1.671	3.42	1.51~5.88	79.85	<0.001
	72	y=2.417x-0.928	2.40	0.60~3.96	61.03	<0.001

x: 植物精油浓度对数值；y: 死亡概率值。x: Logarithm of concentration of plant essential oils; y: probability of death.

2.2 4种植物精油对小菜蛾幼虫的拒食活性

4种植物精油对小菜蛾3龄幼虫表现出不同程度的拒食活性, 拒食率与精油浓度之间无直接联系, 但在相同浓度下, 选择性拒食率高于非选择性拒食率(图1)。7.5 μg/μL 辣蓼植物精油处理时, 小菜蛾3龄幼虫的选择拒食率最高, 作用24 h和48 h后分别为89.28%和76.67%(图1-A), 而5 μg/μL 浓度作用24 h和浓度10 μg/μL 作用48 h时非选择拒食率最高, 分别为62.66%和55.00%(图1-B)。5 μg/μL 迷迭香植物精油处理时, 小菜蛾3龄幼虫的拒食作用最好, 作用24 h和48 h时, 选择拒食率分别为82.87% 和

69.96%(图1-C), 而15 μg/μL 浓度作用24 h和浓度5 μg/μL 作用48 h时非选择拒食率最高, 分别为64.19%和36.60%(图1-D)。15 μg/μL 芸香植物精油处理时, 小菜蛾3龄幼虫的拒食活性最好, 作用24 h和48 h时选择拒食率分别为100.00% 和92.84%(图1-E), 10 μg/μL 浓度作用24 h和48 h时非选择拒食率最高, 分别为65.72%和42.72%(图1-F)。15 μg/μL 紫苏植物精油作用24 h和浓度10 μg/μL 作用48 h时拒食效果最明显, 选择拒食率分别为100.00% 和85.35%(图1-G), 15 μg/μL 浓度作用24 h和48 h时非选择拒食率最高, 分别为59.88%和55.36%(图1-H)。

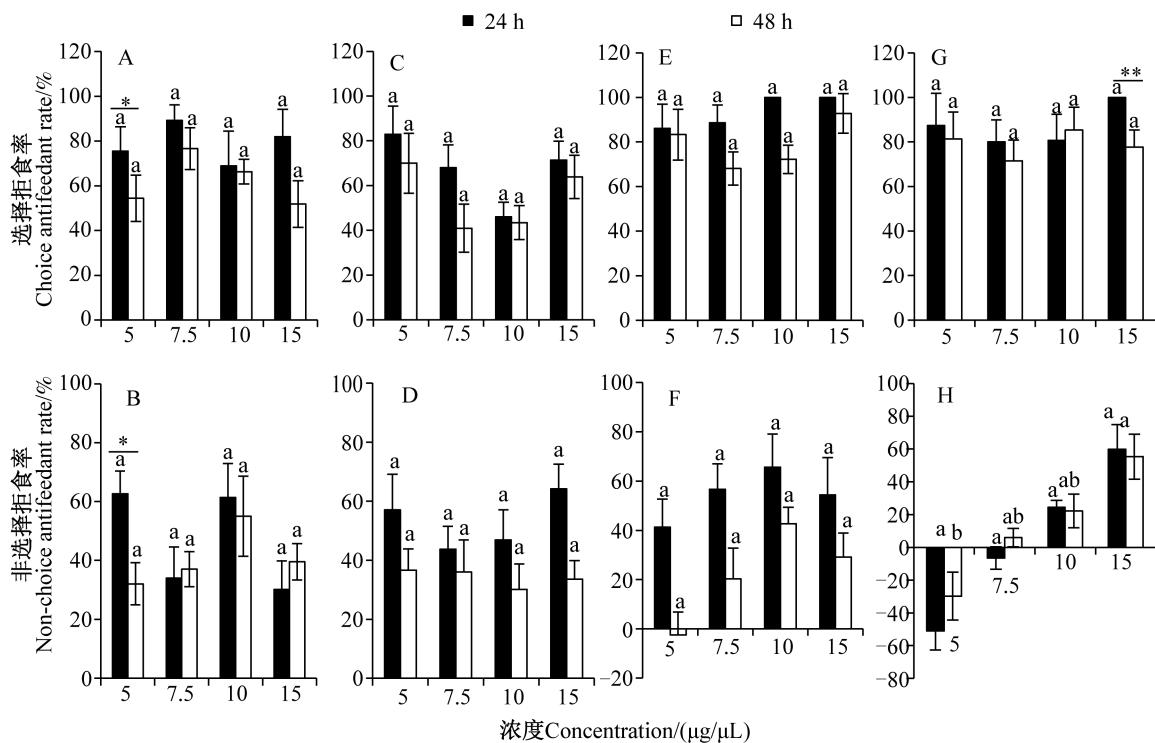


图1 不同浓度辣蓼(A~B)、迷迭香(C~D)、芸香(E~F)和紫苏(G~H)植物精油对小菜蛾幼虫的拒食作用

Fig. 1 Antifeedant effects of different concentrations of essential oils from *Polygonum hydropiper* (A-B), *Rosmarinus officinalis* (C-D), *Ruta graveolens* (E-F) and *Perilla frutescens* (G-H) on *Plutella xylostella* larvae

图中数据均为平均数±标准误。同色柱不同小写字母表示经Tukey's HSD检验在P<0.05水平差异显著。*、**分别表示同浓度下不同时间之间经Student's t测验法检验在P<0.05和P<0.01水平差异显著。Data are mean±SE. Different lowercase letters on the bars of the same color indicate significant difference at P<0.05 level by Tukey's HSD test. *, ** indicate significant difference between different time points at the same concentration at P<0.05 and P<0.01 levels by Student's t test, respectively.

2.3 4种植物精油对小菜蛾幼虫的生长抑制活性

随着浓度的升高, 4种植物精油对小菜蛾3龄幼虫的相对生长率降低, 即相对生长率与精油浓度基本呈负相关关系; 随着浓度升高, 4种植物精油对小菜蛾3龄幼虫的生长抑制率升高, 即生长抑制率与精油浓度基本呈正相关关系(图2)。在4种植物精油中, 芸香植物精油对3龄幼虫的生长抑制作用最明显, 相对生长率最低为-34.72%, 生长抑制率最高

为133.74%; 辣蓼植物精油的生长抑制效果次之, 相对生长率最低为-15.13%, 生长抑制率最高为107.67%; 迷迭香和紫苏植物精油的生长抑制效果相对较差(图2)。

2.4 4种植物精油对小菜蛾成虫的EAG反应

小菜蛾雌、雄成虫对不同植物精油的不同浓度能产生不同程度的EAG反应, 且反应值与精油浓度基本呈正相关关系(图3)。在相同浓度下, 辣蓼和

芸香植物精油引起的小菜蛾雌成虫EAG值均显著高于雄成虫(图3-A、3-C),而迷迭香和紫苏植物精油引起的小菜蛾雌、雄成虫EAG值之间无显著差异。

(图3-B、3-D)。在500 μg/μL浓度下,芸香、辣蓼、紫苏和迷迭香植物精油引起的雌成虫电位反应均达到峰值,分别为0.80、0.74、0.51和0.44 mV。

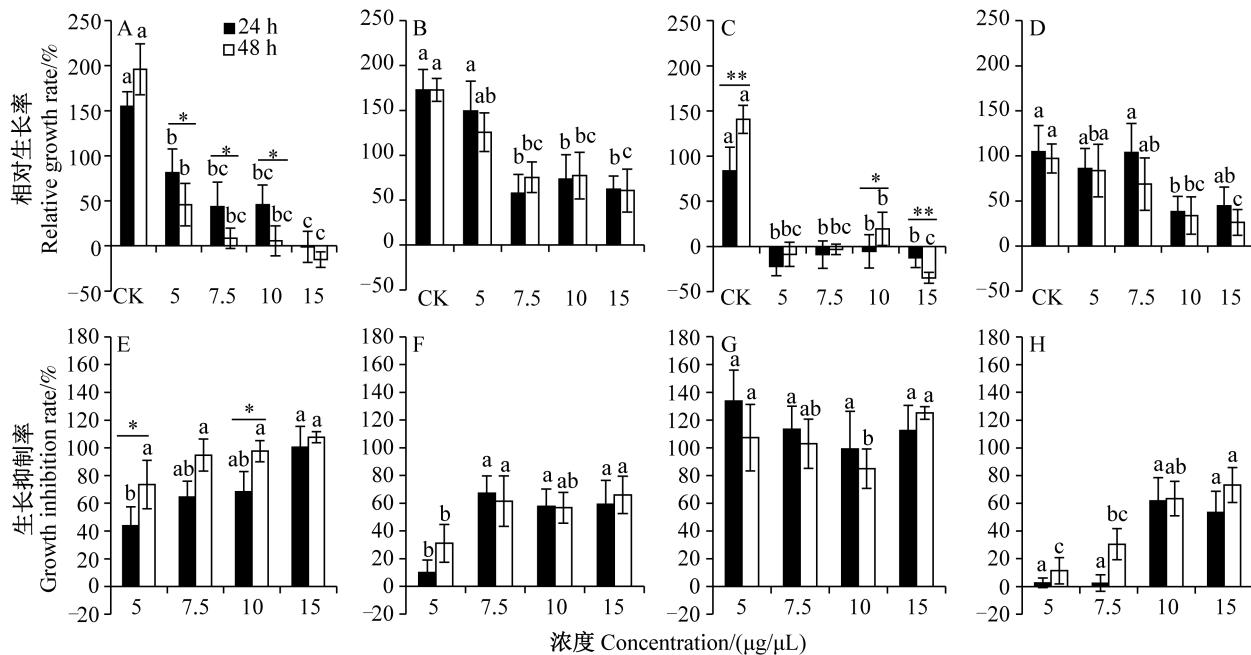


图2 不同浓度辣蓼(A, E)、迷迭香(B, F)、芸香(C, G)和紫苏(D, H)植物精油对小菜蛾相对生长率和生长抑制率
Fig. 2 Relative growth rates and the growth inhibition rates of *Plutella xylostella* under different concentrations of essential oils from *Polygonum hydropiper* (A, E), *Rosmarinus officinalis* (B, F), *Ruta graveolens* (C, G) and *Perilla frutescens* (D, H)

图中数据均为平均数±标准误。同色柱不同小写字母表示经Tukey's HSD检验在P<0.05水平差异显著。*、**分别表示同浓度下不同时间之间经Student's t测验法检验在P<0.05和P<0.01水平差异显著。Data are mean±SE. Different lowercase letters on the bars of the same color indicate significant difference at P<0.05 level by Tukey's HSD test. *, ** indicate significant difference between different times at the same concentration at P<0.05 and P<0.01 levels by Student's t test, respectively.

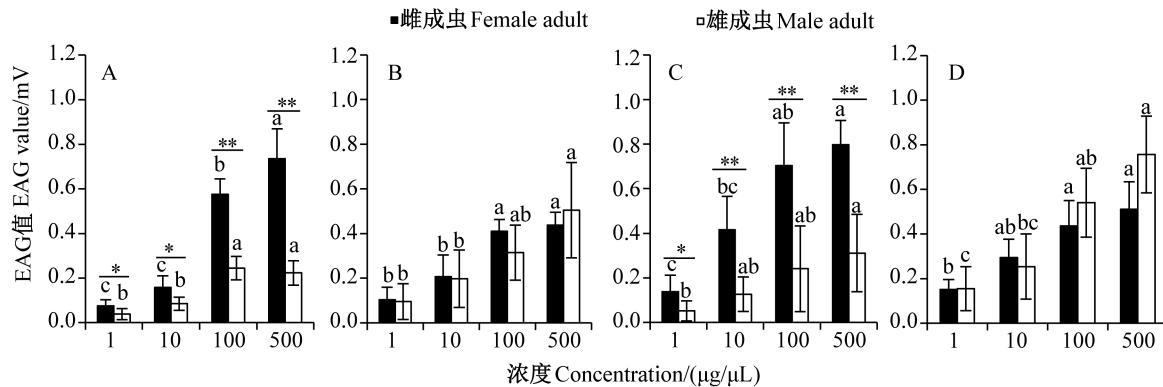


图3 小菜蛾雌、雄成虫对不同浓度辣蓼(A)、迷迭香(B)、芸香(C)和紫苏(D)植物精油的触角电位反应

Fig. 3 Electroantennographic responses of *Plutella xylostella* female and male adults to different concentrations of plant essential oils from *Polygonum hydropiper* (A), *Rosmarinus officinalis* (B), *Ruta graveolens* (C) and *Perilla frutescens* (D)

图中数据均为平均数±标准误。同色柱不同小写字母表示经Tukey's HSD检验在P<0.05水平差异显著。*、**分别表示同浓度下雌、雄成虫之间经Student's t测验法检验在P<0.05和P<0.01水平差异显著。Data are mean±SE. Different lowercase letters on the bars of the same color indicate significant difference at P<0.05 level by Tukey's HSD test. *, ** indicate significant difference between female and male adults at the same concentration at P<0.05 and P<0.01 levels by Student's t test, respectively.

2.5 4种植物精油对小菜蛾成虫的嗅觉行为反应

小菜蛾雌、雄成虫对4种植物精油气味均表现

出一定的驱避活性,且精油种类和浓度不同,驱避效果不同。1、100、500 $\mu\text{g}/\mu\text{L}$ 辣蓼植物精油对小菜蛾

雌成虫驱避效果显著; 10~500 $\mu\text{g}/\text{mL}$ 辣蓼植物精油对雄成虫有显著驱避作用(图4-A)。1、10、100 和 500 $\mu\text{g}/\text{mL}$ 迷迭香植物精油对雌成虫的驱避作用显著; 10 $\mu\text{g}/\text{mL}$ 和 100 $\mu\text{g}/\text{mL}$ 迷迭香植物精油对雄成虫有极显著的驱避作用(图4-B)。10~500 $\mu\text{g}/\text{mL}$ 芸香

植物精油对雌成虫有极显著的驱避作用; 1~100 $\mu\text{g}/\text{mL}$ 芸香植物精油对雄成虫的驱避效果也极显著(图4-C)。1 $\mu\text{g}/\text{mL}$ 和 100 $\mu\text{g}/\text{mL}$ 紫苏植物精油对雌成虫有极显著的驱避作用; 10~500 $\mu\text{g}/\text{mL}$ 紫苏植物精油对雄成虫的驱避效果也极显著(图4-D)。

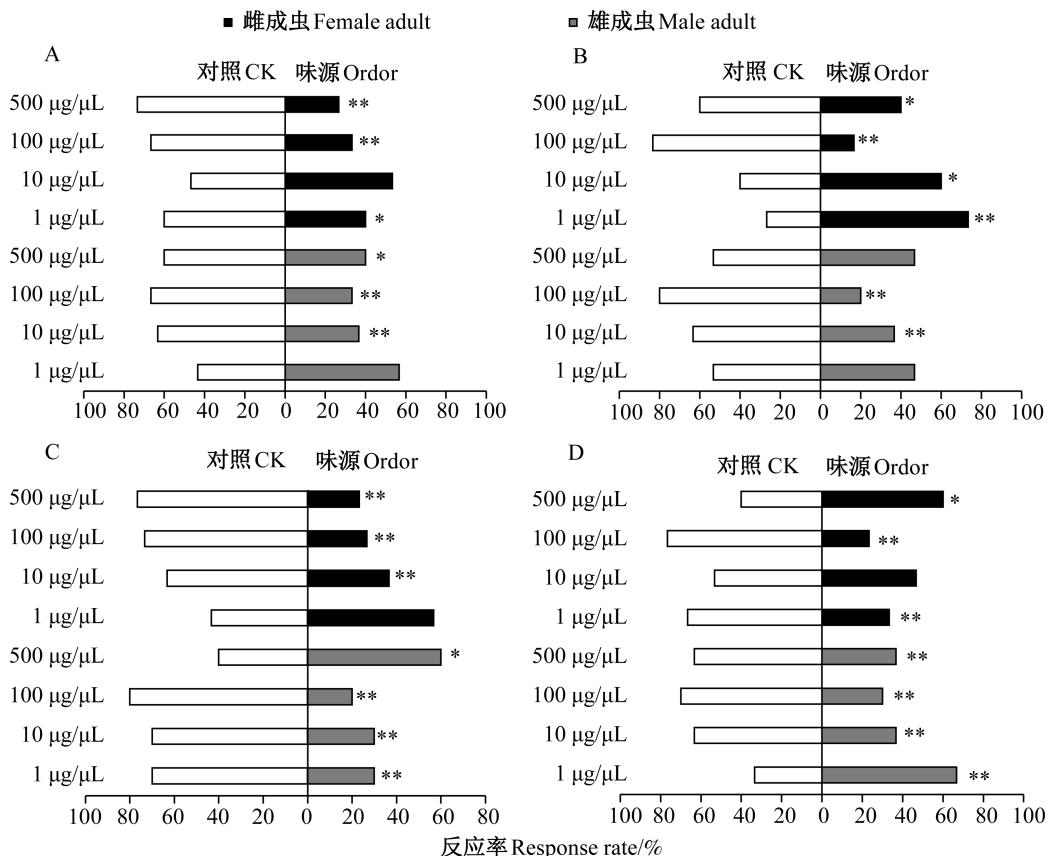


图4 小菜蛾雌、雄成虫对不同浓度辣蓼(A)、迷迭香(B)、芸香(C)和紫苏(D)植物精油的行为反应

Fig. 4 Behavioral responses of *Plutella xylostella* female and male adults to different concentrations of plant essential oils from *Polygonum hydropiper* (A), *Rosmarinus officinalis* (B), *Ruta graveolens* (C) and *Perilla frutescens* (D)

*、**分别表示对照与处理之间经卡方检验在 $P<0.05$ 和 $P<0.01$ 水平差异显著。*, ** indicate significant differences between treatment and the control at $P<0.05$ and $P<0.01$ level by χ^2 test, respectively.

2.6 芸香植物精油的GC-MS分析

芸香植物精油共鉴定出38种化合物。萜类及其氧化物是芸香植物精油的主要成分, 其中单萜类物质有9种, 含氧单萜类7种, 倍半萜类6种, 含氧倍半萜类2种。精油中 β -石竹烯的含量最高, 其次是(+)- α -蒎烯、 β -蒎烯、左旋乙酸冰片酯和3-苯丙醇(表2)。

3 讨论

植物精油主要通过干扰靶标害虫的行为和生理来实现对害虫的调控(Isman, 2006)。本研究测定了4种常见植物精油对小菜蛾幼虫和成虫的生物活性, 结果显示辣蓼植物精油对小菜蛾幼虫表现出显

著的毒杀效果。李强等(2006)研究结果也表明辣蓼植物精油对小菜蛾成虫有较好的熏蒸活性, 处理24、48、72 h后LC₅₀分别为6.30、5.42和5.12 mg/mL。因此, 推测辣蓼植物精油对小菜蛾幼虫和成虫均有毒杀作用, 这可能是由于辣蓼包含具有抗微生物、抗氧化、抗病毒、杀虫等生物活性的黄酮类和倍半萜类化学成分(姚祖凤等, 1999; 曾维爱等, 2006)。Ayaz et al.(2016b)在GC-MS分析中也发现辣蓼粗提物中有法尼醇、棕榈酸甲酯和肉豆蔻酸等已报道的杀虫活性化合物。除此之外, 芸香植物精油对幼虫也有良好的毒杀作用, 这可能是由于芸香包含多种杀虫和抑菌活性化合物(吴娇等, 2015)。

表2 芸香植物精油成分 GC-MS 分析

Table 2 GC-MS analysis of essential oils from *Ruta graveolens*

序号 No.	化合物 Compound	CAS号 CAS number	分子式 Molecular formula	保留时间 Retention time/min	相对含量 Relative content/%
1	(+)- α -蒎烯 (+)- α -pinene	7785-70-8	$C_{10}H_{16}$	5.63	5.44
2	莰烯 Camphene	79-92-5	$C_{10}H_{16}$	6.40	2.09
3	苯甲醛 Benzaldehyde	100-52-7	C_7H_6O	6.98	0.07
4	桧烯 Sabinene	3387-41-5	$C_{10}H_{16}$	7.49	0.16
5	β -蒎烯 β -pinene	127-91-3	$C_{10}H_{16}$	7.70	4.71
6	月桂烯 β -myrcene	123-35-3	$C_{10}H_{16}$	8.29	0.19
7	松油烯 α -terpinene	99-86-5	$C_{10}H_{16}$	9.55	0.05
8	(+)-柠檬烯 (+)-limonene	5989-27-5	$C_{10}H_{16}$	10.12	1.72
9	桉叶油醇 1,8-cineole	470-82-6	$C_{10}H_{18}O$	10.25	0.03
10	γ -松油烯 γ -terpinene	99-85-4	$C_{10}H_{16}$	11.44	0.49
11	苯乙酮 Acetophenone	98-86-2	C_8H_8O	11.74	0.11
12	萜品油烯 Terpinolene	586-62-9	$C_{10}H_{16}$	12.63	0.07
13	芳樟醇 Linalool	78-70-6	$C_{10}H_{18}O$	13.31	0.08
14	(\pm)樟脑 (\pm)-camphor	464-49-3	$C_{10}H_{16}O$	15.22	0.54
15	(+)-异薄荷酮 (+)-isomenthone	1196-31-2	$C_{10}H_{18}O$	15.60	0.01
16	苯丙醛 Phenylpropyl aldehyde	104-53-0	$C_9H_{10}O$	15.86	0.02
17	2-莰醇 Borneol	507-70-0	$C_{10}H_{18}O$	16.27	1.05
18	(-)-4-萜品醇 (-)-terpinen-4-ol	20126-76-5	$C_{10}H_{18}O$	16.60	0.09
19	α -松油醇 α -terpineol	98-55-5	$C_{10}H_{18}O$	17.20	0.25
20	3-苯丙醇 3-phenyl-1-propanol	122-97-4	$C_9H_{12}O$	18.53	2.44
21	L-香芹酮 Carvone	99-49-0	$C_{10}H_{14}O$	19.03	0.10
22	肉桂醛 Cinnamaldehyde	104-55-2	C_9H_8O	20.15	0.06
23	左旋乙酸冰片酯 l-bornyl acetate	5655-61-8	$C_{12}H_{20}O$	20.59	2.50
24	肉桂醇 Cinnamyl alcohol	104-54-1	$C_9H_{10}O$	21.61	0.77
25	3-苯丙酸乙酯 Ethyl 3-phenylpropanoate	2021-28-5	$C_{11}H_{14}O_2$	22.82	2.17
26	(+)-环苜蓿烯 (+)-cyclosativene	22469-52-9	$C_{15}H_{24}$	23.29	0.31
27	(-) α -蒎烯 α -copaene	3856-25-5	$C_{15}H_{24}$	23.43	0.24
28	β -石竹烯 β -caryophyllene	87-44-5	$C_{15}H_{24}$	24.2	31.17
29	香豆素 Coumarin	91-64-5	$C_9H_6O_2$	24.54	0.05
30	(-) α -荜澄茄油烯 (-) α -cubebene	17699-14-8	$C_{15}H_{24}$	24.6	0.07
31	α -律草烯 α -caryophyllene	6753-98-6	$C_{15}H_{24}$	24.7	0.72
32	肉桂酸乙酯 Ethyl cinnamate	103-36-6	$C_{11}H_{12}O_2$	24.83	1.33
33	大根香叶烯 D Germacrene D	23986-74-5	$C_{15}H_{24}$	25.02	1.77
34	石竹素 Caryophyllene oxide	1139-30-6	$C_{15}H_{24}O$	26.07, 26.79	1.54
35	二十酸 Arachidic acid	506-30-9	$C_{20}H_{40}O_2$	27.74	0.65
36	β -桉叶醇 β -eudesmol	473-15-4	$C_{15}H_{26}O$	27.91	0.91
37	肉桂酸苄酯 Benzyl cinnamate	103-41-3	$C_{16}H_{14}O_2$	30.51	0.36
38	木蜡酸甲酯 Methyl tetracosanoate	2442-49-1	$C_{25}H_{50}O_2$	30.64	0.94

大多数具有防御性化学物质的天然植物通过干扰或抑制害虫的取食或产卵行为,进而损害幼虫生长而非杀死害虫来防止草食性昆虫的侵害。这些化学物质与嗅觉蛋白结合,作用于昆虫触角或口器部位的嗅觉受体神经元,进而对其取食和生长产生一定的影响(Yang et al., 2017)。本研究结果发现芸香精油对小菜蛾的拒食效果最明显,不同植物精油对昆虫拒食程度不同,可能与精油中特殊活性物质的含量有关(张正群等,2014)。张正群等(2012)研究结果显示,芸香提取液对茶尺蠖的非选择拒食作用

显著,而选择性拒食效果较差;而本研究发现芸香植物精油对小菜蛾的选择性拒食和非选择性拒食都有显著作用,尤其当浓度为 15 μ g/ μ L 时,作用 24 h 和 48 h 时的选择性拒食率分别高达 100.00% 和 92.84%。由此推测,相较于茶尺蠖,芸香植物精油对小菜蛾的拒食作用更显著。但是植物提取物在应用过程中最棘手的问题之一是它的有效成分随作用时间的增加而减少,且害虫能在较短的时间内适应拒食剂(李水清等,2005),而本研究表明,芸香植物精油作用 24 h 对小菜蛾幼虫的拒食率与作用 48 h 的

基本无显著差异,拒食效果稳定,这可能是由于芸香植物精油中的特殊化合物对小菜蛾幼虫的感受受体具有较强的刺激作用,也可能是这些特殊化合物对幼虫体内的保护酶和解毒酶等有较强的抑制作用。

植物精油在引起昆虫拒食行为的同时,也会延长幼虫生长期,增加死亡率,引起非正常变态,减少产卵量,降低化蛹率和孵化率等(曾维爱,2007)。本研究结果显示,芸香植物精油对小菜蛾幼虫有显著的生长抑制作用;张正群等(2012)研究发现芸香提取液处理后茶尺蠖幼虫对食物的转化率仅为5.08%,与本研究结果一致。这种现象一方面可能是由于被测幼虫的拒食率增加,获得的食物量减少,从而抑制了昆虫的生长发育(Pavela, 2011);另一方面可能是因为芸香精油进入试虫体内,引起昆虫脱水,破坏中肠组织,影响消化,干扰呼吸代谢,阻断正常生理生化反应,从而抑制了昆虫的生长发育。

昆虫触角上分布的多个嗅觉受体蛋白可与不同的信息化合物结合,产生的嗅觉信号经中枢神经系统加工和整合后调节昆虫的行为(徐伟等,2018)。本研究测试了4种植物精油对小菜蛾雌、雄成虫的电生理反应,结果显示这4种植物精油在各测试浓度下均能引起小菜蛾雌、雄成虫的电生理反应,但敏感程度有差异,这可能是由于不同精油的活性物质与小菜蛾嗅觉感受蛋白结合能力不同,进而引起的电位反应值有所差异。芸香和辣蓼植物精油在相同浓度下引起雌成虫的触角EAG值均显著高于雄成虫,当芸香植物精油浓度为100 μg/μL时,雌成虫EAG值是雄成虫的2.91倍,当浓度为500 μg/μL时,雌成虫EAG值是雄成虫的2.58倍。同时,当辣蓼植物精油浓度为500 μg/μL时,雌成虫EAG值是雄成虫的2.96倍,究其原因可能是小菜蛾雌雄成虫在寄主定位、求偶和寻找产卵位点等行为过程中分工不同,也可能是小菜蛾雌成虫触角上的锥形和腔椎形感器较雄成虫丰富(闫喜中,2014)。然而,EAG值只能判断植物精油对昆虫刺激作用的大小,其作用机理及具体作用感器还有待进一步研究。

本研究结果显示4种植物精油均能引起小菜蛾不同程度的驱避行为。Thompson(1988)和Lu et al.(2004)经典观点认为,非寄主挥发物对植食性昆虫具有驱避作用,与本研究结果一致。这些植物及提取物对其他害虫也有一定的驱避效果,如张正群(2014)发现迷迭香植物精油对茶尺蠖有显著的驱避作用;袁莲莲等(2016)发现紫苏乙醇提取物对烟蚜*Myzus persicae*的忌避活性较好,忌避率在7.34%~

98.35%之间。本研究结果还显示,芸香植物精油对小菜蛾的驱避作用最显著;Hadis et al.(2003)研究发现人腿上涂抹芸香提取物后对曼索尼娅种群(双翅目蚊科)具有较好的驱避作用;杨频(2004)研究发现在7%浓度下芸香植物精油对白蚊伊蚊*Aedes albopictus*同样具有驱避作用,与本研究结果一致,表明芸香植物精油具有制备成为小菜蛾行为调节剂的潜在价值。

本研究的GC-MS分析结果显示,芸香植物精油主要成分为 β -石竹烯、(+)- α -蒎烯和 β -蒎烯等,与Rojht et al.(2012)研究结果相似,但化合物比例和次要成分存在差异。而Chaabani et al.(2019)发现芸香植物精油中含有丰富的烃类酮(52.5%),主要是1-壬烯(19.4%)、2-十一烷酮(16.22%)和2-壬酮(11.9%),与本研究结果存在差异,这可能是由于植物精油的化学成分和含量主要受基因型和农艺条件的影响(Reddy et al., 2016)。刘雨晴等(2010)研究表明, β -石竹烯和 α -蒎烯对棉蚜*Aphis gossypii*的触杀毒力都较高,并以 β -石竹烯的毒性最强。其次,芸香植物精油中含量较少的成分,如月桂烯,对茶尺蠖也有显著的驱避作用(Zhang et al., 2015)。芸香植物精油对小菜蛾的生物活性是否与主成分 β -石竹烯、蒎烯以及次要成分月桂烯等物质有关,有待进一步验证。

植物精油是从植物中提取的次生混合物,由于其具有多种生物活性,可作为开发为害虫绿色防控剂的重要资源。本试验结果表明芸香植物精油在小菜蛾防治中具有重要价值。芸香植物精油和其主要成分对害虫寿命、结蛹率和羽化率等的影响,以及在田间防治中的应用技术还有待进一步研究。

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