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RESEARCH **P**APER

Effect of andrographolide on feeding behaviour of *Papilio demoleus* L., (Lepidoptera: papilionidae) larvae

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Botanical insecticides are currently of interest because of their successful application in plant protection as biocontrol agents. Andrographolide which was isolated from *Andrographis paniculata* evaluated for its antifeedant activity against the fourth-instar larvae of *Papilio demoleus*. Preliminary screening after 24 hrs and 48 hrs of exposure at a concentration of 200 ppm exhibited 83.60 per cent and 80.05 per cent antifeedant activity significantly. The protected leaf area at 200ppm was 32.08 ± 0.84 sq.cm and 28.55 ± 0.72 sq.cm after 24 hrs. and 48 hrs, respectively. The antifeedant activity of andrographolide was also examined at 150, 100 and 50ppm concentrations which significantly reduced the feeding ability of larvae. The results imply that extract of *A. paniculata*, can potentially be used as an alternative pest control agent against the *Papilio demoleus* larvae.

Key words : Antifeedant activity, Photochemical, Andrographolide, *Papilio demoleus* larvae, Pest control

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INTRODUCTION

Since the dawn of civilization, the man has been able to improve his living condition by practicing agriculture (Gupta, 2004). Therefore, the control of insects, weeds, fungi and other pests of cultivated crops for food and fodder has been always a challenge to protect essential food sources. One of the ways to control crop damage by infestation of pests is to use insecticides either synthetic or natural. It is obviously important that these insecticides should not be toxic to non-target organisms and the environment. Developed and developing countries progressing in agriculture sector, by introducing various kinds of pesticides into the field for improving yields and thus face serious pollution challenges, pest resurgences, pest resistance to pesticides and lethal effect to non-target organisms in the agro-ecosystems in addition to direct toxicity to users.

Therefore, in order to search an environmentally safe alternative, scientists considered the pesticides of biological origin (biopesticides) in the place of synthetic insecticides. Replacement of synthetic insecticides by plant products is an universally acceptable and practicable approach. Plant natural products have enormous potential to inspire and influence modern agrochemical research (Benner, 1993). The plant kingdom can be a rich source of a variety of chemicals with the potential for development as successful pest control agents (Arnason *et al.*, 1989; Rahman *et al.*, 1999). Secondary compounds from plants include alkaloids, terpenoids, phenolics, flavonoids, chromenes and other minor chemicals can affect insects in several ways. They may disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phago-stimulants antifeedants or modify oviposition. They may also retard or accelerate development or interfere with the life cycle

of the insect in other ways (Raja *et al.*,2001; Papachristos and Stamopoulos, 2002; Tapondjou *et al.*, 2002).

The Lemon butterfly *Papilio demoleus* L. (Lepidoptera: Papilionidae), is a serious pest on citrus, distributed throughout the tropical and subtropical parts of the world including India, Pakistan, Japan, China and South East Asia. *Papilio demoleus* L. has been reported as an increasingly important pest during the rainy seasons causing heavy yield loss throughout India. The larvae of this pest are vigorous foliage feeders. In cases of severe infestation, young seedlings get completely defoliated. To overcome this, large quantities of insecticides have been used (Qin *et al.*, 2000; Shi *et al.*, 2003; Khetagoudar *et al.*, 2012). In the present study an attempt has been made to evaluate the antifeedant activity of andrographolide against fourth instar larvae of *Papilio demoleus* L.

RESEARCH METHODOLOGY

Test insect :

The *Papilio demoleus* Linnaeus, is also called as citrus swallowtail, ranges widely and is an extremely successful invader. The larvae are a serious pest of citrus nursery stock and other young citrus trees, Rutaceae family in Asia and the Middle East. First instars are black in colour with a black head, with two sub-dorsal rows of short fleshy spines. Second, third, and fourth instars have a dark brown and glossy head capsule. Fifth instars are cylindrically shaped and tapered anteriorly (Plate A). The pupal duration is variable. In some areas, it is about 30 days in spring, reducing to 18 days in summer. This species typically has five instars and is capable of producing multiple generations per year depending on temperature constraints. Near the equator as many as nine generations may be seen. The average length of a generation varies from 26 to 59 days (Ravindranathan, 2003).

Collection of larvae :

Egg batches and different developmental stage instars of *Papilio demoleus* larvae were collected from cultivated farm fields near Palem village, near Nakrekal town of Nalgonda district, Telangana, India and reared in the laboratory on leaves of citrus at room temperature (25 ±3°C). The fourth instar larvae were preferred for the experiment as they are voracious feeders.

Plant material :

Andrographis paniculata is an herbaceous plant

Ist, 2nd and 3rd Instars

4th and 5th Instars

Adult

Plate A : Different stages of *Papiplio demoleus*

of family Acanthaceae, genus *Andrographis* native to India and Srilanka. It is widely cultivated in southern Asia (Sharm and Joshi, 2011). The plant extract exhibits a wide spectrum of biological activities including antimicrobial, antimalarial, antihypertensives and antidiabetics, antifilarial (Manvitha Mohan *et al.,* 2013). Antifeedant properties, antioviposition properties (Hermawan *et al.,* 1994), larvicidal activity (Ramya *et al.,* 2011), malaria and respiratory infections (Koteswara Rao *et al.,* 2004), inhibit human breast, liver and prostate cancer cells (Basak *et al.,* 2008).

Antifeedant activity bioassy :

Antifeedant bioassay was studied using leaf disc non-choice method, The fresh leman leaf discs of 36.5sq.cm in diameter were punched using cork borer and dipped in 50 ppm, 100ppm, 150ppm and 200 ppm concentrations of andrographolide. Andrographolide was used as positive control, leaf discs treated with acetone was considered as negative controls. In each Petridish, a wet filter paper was placed to avoid the early drying of the leaf discs and single 2 hrs pre-starved fourth instar larva of *Papilio demoleus* was introduced into Petridishes containing the respective leaf discs. Progressive consumption of leaf area by the treated and control larvae after 24 hrs and 48 hrs were recorded using leaf area meter. Leaf area, eaten by larvae in treatment was corrected from the negative control. Ten replicates were maintained for each treatment. The percentage of antifeedant index was calculated using the following formula (Ben Jannet *et al.,* 2000).

$$\text{Antifeedant activity} = \frac{\text{Leaf area consumed in control} - \text{Leaf area consumed in treatment}}{\text{Leaf area consumed in control} + \text{Leaf area consumed in treatment}} \times 100$$

Statistical analysis :

For statistical analysis efficacy of andrographolide on feeding deterrence of *Papilio demoleus*, with various concentrations was analyzed using the programme SPSS 12.

RESEARCH FINDINGS AND ANALYSIS

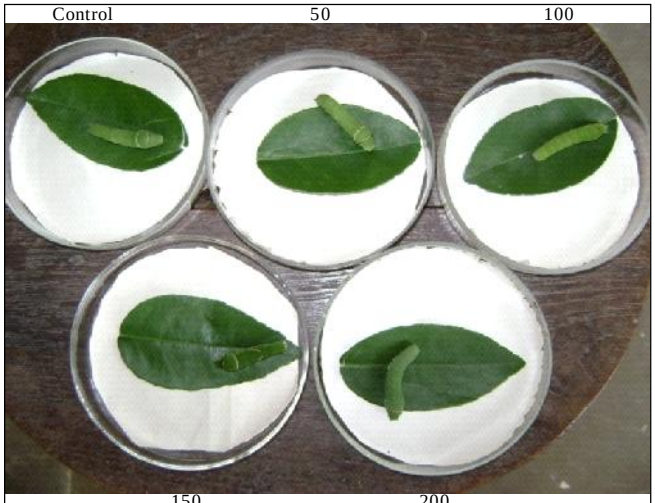
The extract andrographolide was applied on the leaf surface using different concentrations such as 200ppm, 150ppm, 100ppm, 50ppm for 24 hrs and 48 hrs duration. The results of present study showed 83.60 per cent and 80.05 per cent antifeedant activity at 200ppm concentration after 24 hrs and 48 hrs exposure, respectively. The protected leaf area at 200 ppm was 32.08 ± 0.84 sq.cm and 28.55 ± 0.72 sq.cm after 24 hrs. and 48 hrs, respectively. At 150ppm concentration the antifeedant activity was 67.59 per cent and 61.27 per cent for 24 hrs and 48 hrs exposure and the protected leaf area was 26.93 ± 1.16 sq.cm and 24.46 ± 1.34sq.cm after 24 hrs. and 48 hrs., respectively. At 100ppm concentration 58.86 per cent and 53.78 per cent antifeedent activity was observed for 24 hrs. and 48 hrs. exposure and protected leaf area was 23.51 ± 1.15sq.cm and 20.63 ± 0.99sq.cm after 24 hrs. and 48 hrs., respectively. Lower dose of 50ppm concentration reduced the larval feeding up to a large extent that was 51.86 per cent after 24 hrs. and 45.68 per cent after 48 hrs. and the protected leaf area was 20.81 ± 0.70 sq.cm and 16.07 ± 1.20sq.cm after 24 hrs. and 48 hrs, respectively (Table 1 and Plate 1). For different plant materials, various effects such as antifeedant activities, growth inhibition, and insecticidal activities have been reported by several others (Giordano *et al.,* 2001; Krishna *et al.,* 2003; Gholamreza, 2014).

In the present study the andrographolide, reduced the feeding rate of *Papilio demoleus* and the rate of feeding significantly varied depending on the concentration. This indicates that the andrographolide inhibited larval feeding behaviour. Antifeedant activity

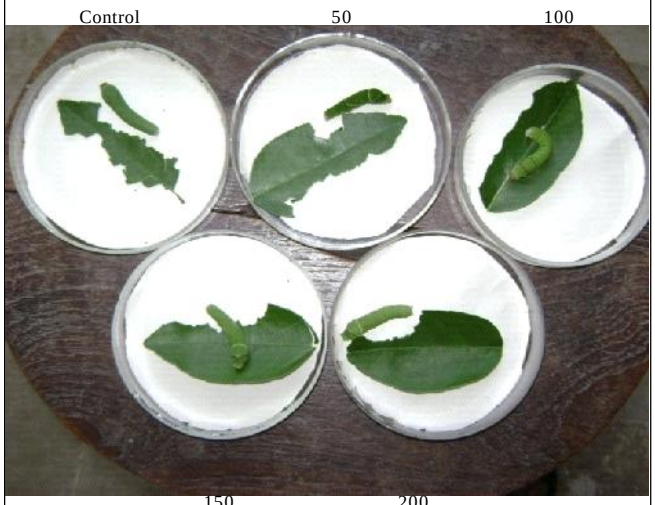
Table 1 : Mean and SD of undamaged leaf area (sq.cm) and antifeedant activity (%) with different concentration treatments of andrographolide

Conc. in ppm	No. of insects	Mean ± SD after 24 hrs	Mean ± SD after 48 hrs	Antifeedant activity (%) after 24hrs	Antifeedant activity (%) after 48hrs
200	10	32.08 ± 0.84*	28.55 ± 0.72*	83.60	80.05
150	10	26.93 ± 1.16*	24.46 ± 1.34*	67.59	61.27
100	10	23.51 ± 1.15*	20.63 ± 0.99*	58.86	53.78
50	10	20.81 ± 0.70*	16.07 ± 1.20*	51.86	45.68
Control	10	18.54 ± 0.74*	10.30 ± 0.91*	–	–

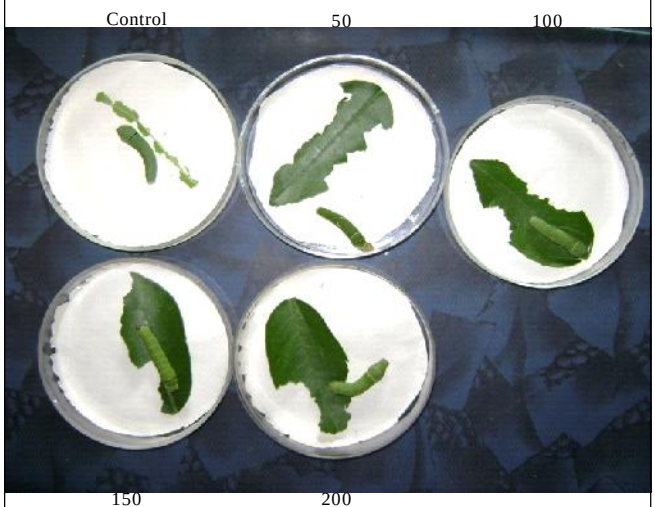
All the treatments showed statically significant compared with control, One way ANOVA, P<0.05



a) Zero hours



b) After 24 hours



c) After 48 hours

Plate 1 : Antifeedant activity of andrographolide against 4th Instar larva of *Papilio demoleus*

will be regarded as inhibition by olfactory and gustatory responses and not by inhibition of a insect feeding and metabolism. Insect feeding deterrents may be perceived either by stimulation of specialized deterrent receptors, which perceive phagostimulating compounds. Isolation and structure elucidation of the andrographolide is important not only for understanding the ecological aspects of insect pest's relationship, but also for their potential in insect pest's control. Food selection and the choice of food is primarily based upon contact chemoreception of various allelochemicals. In particular, dietary experience has influenced the ability of insects to taste plant chemicals that may have served as signals of suitability or unsuitability. The findings of the present study are in agreement with the earlier reports of (Nishida *et al.,* 2004; Gonzalez-Coloma *et al.,* 2005 and Jeyasankar *et al.,* 2014).

Azadirachtin, a terpenoid isolated from the neem tree, stimulates a deterrent receptor in a number of herbivorous insects. Several investigators have already reported that botanicals offer antifeedant activity against *Spodoptera litura* (Ulrichs *et al.,* 2008; Sreelatha *et al.,* 2010; Arivoli *et al.,* 2012). Mikolajczak and Reed (1987) stated that the seed extracts of *Trichilia prieureana*, *Trichilia roka* and *Trichilia connaraides* exhibited high levels of antifeedant activity in leaf disc method against *Spodoptera frugiperda*. The extract of *Adhatoda vasica* leaves was found to have feeding deterrent properties when applied on leaf disc method (Sadek *et al.,* 2003). The results of the present study are also supported by the studies of Kumari *et al.* (2003), who isolated neoclerodane diterpenoids from *Clerodendron* spp. for effective control of *S. litura*, Cifuente *et al.* (2002) studied the antifeedant activity of clerodane diterpenoides from *Baccharis sagittalis*. and reported significant antifeedant activity against *Tenebrio molitor* using a non- choice disc method.

Different feeding inhibitors cause different changes in the physiological mechanisms operating at each level of larval digestive system. A preingestive inhibitor that affects chemosensory cells in sensillas on antennae and causes reduced palpation while a postingestive inhibitor targeting midgut muscles may cause gut movements. Post-ingestive inhibition reactions take longer time and produce more chronic effects.

Conclusion :

It can be concluded that andrographolide has the

potential for use in the management of *Papilio demoleus*. The antifeedant effects together would have synergistic effect in restricting population build-up of *Papilio demoleus* in the field. Moreover, this method of management would be economical, sustainable and ecofriendly in nature.

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