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AZADIRACHTA INDICA-LEAVES, ALLIUM SATIVUM-BULBS AND OSCIMUM
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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND THE ANTIFEEDANT EFFECT OF AZADIRACHTA INDICA-LEAVES, ALLIUM SATIVUM-BULBS AND OSCIMUM SANCTUM-LEAVES ON THE LARVAE OF CHILO PARTELLUS

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ABSTRACT

The present study deals with the antifeedant effect of different plant extracts on Maize stem borer, *Chilo partellus*. Three medicinal plant material like *Azadirachta indica*-Leaves, *Allium sativum*-Bulbs and *Oscimum sanctum*- Leaves extract were tested against Maize stem borer, *Chilo partellus* (Swinhoe). The plant extract was prepared by soxhlet extraction method using two solvents like distilled water and ethanol at different percent concentrations (2.0 %-10 %) were used. Phytochemical screening were conducted using standard methods of analysis and the antifeedant effect of plant extract were carried out by using leaf disk choice bioassays. The result of the phytochemical screening was done to check the presence of flavonoids, alkaloids, saponins, tannins and cardiac glycosides. The antifeedant effect of plant extracts were recorded at 12, 24 and 48 hours. All three plant extract in both solvents showed the antifeedant activity. The ethanolic extract of *Azadirachta indica* (leaves) followed by *Allium sativum* (bulbs) were found effective and showed the highest antifeedant effect as compared to *Oscimum sanctum*. 10.0 % concentration of Ethanolic extract of *Azadirachta indica* showed the maximum antifeedant effect (80.33 %) followed by *Allium sativum* (74.37 %) at 48 hours and the minimum antifeedant (2.09 %) was observed in 2 % aqueous extract of *Oscimum sanctum* at 12 hours. The concentration of plant extracts increases, the antifeedant effect also increased. Therefore, in general the antifeedant effect of different concentrations, irrespective of the extracts decreased with decrease in concentration from 10 % to 2 %.

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INTRODUCTION

Maize stem borer, *Chilo partellus* Swinhoe (Lepidoptera:Pyralidae) is one of the major biotic constraints in successful maize and sorghum production worldwide, particularly in Asia and Africa (Arabjafari and Jalali, 2007). Yield losses caused by stem borers could be as high as 80% for maize and as much as 88% in sorghum (Seshu Reddy, 1988). A variety of insecticide chemistries and formulations have been found effective against the pest. The use of insecticides can be environmentally disruptive and can result in elimination of beneficial insects and accumulation of residues in the harvested produce (CIMMYT, 1999). Botanical insecticides have long been considered as attractive alternatives to synthetic chemical insecticides for pest management (Cox, 2002). Botanical pesticides are eco-friendly, economic, target-specific and biodegradable. Their greatest strength is their specificity as

most are essentially nontoxic and non-pathogenic to animals and humans.

Botanical insecticides such as Azadirachtin are often effective alternatives to organophosphates or other neurotoxins for pest control due to multiple modes of action. These include toxicity, antifeedant and anti-oviposition effects (Sutherland *et al.*, 2002). Natural products containing secondary plant compounds such as terpenes, steroids, alkaloids, phenolics and cardiac Glycosides affect insect behavior and are toxic in some cases (Gokce *et al.*, 2009). Identification of plant extracts that exhibit the above-described deleterious effects on pest insect physiology and behavior represents a potential alternative strategy for development of biorational controls that could replace synthetic neurotoxins. Our intent was to identify potential botanical insecticides that may prove to be effective alternatives for controlling *Chilo partellus* (MSB).

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Considering the importance of eco-friendly approaches to manage the pest (MSB), the present experiment was designed to determine relative efficacy of different botanical extracts (*Azadirachta indica*, *Allium sativum* and *Oscimum sanctum*) against *Chilo partellus* (MSB). The high costs of synthetic pesticides and associated toxicity risks discourage to integrate into insect pest management systems (CSA, 2012; Echereobia et al., 2010). So as, to encourage growers to produce natural insecticides in response to the rising cost of synthetic pesticides and to minimise contamination risks caused by chemical insecticide residues in maize crop.

MATERIALS AND METHODS

Study Area

The proposed investigation was done in maize fields of Indore region. The following areas were selected in indore region.

1. Mangaliya
2. Mhow
3. Depalpur
4. Sanwer

Experimental plant

Following plants were selected.

Azadirachta indica (Neem)

Allium sativum (Garlic)

Ocimum sanctum (Tulsi)

Collection of plant materials

Selected plant materials i.e. leaves of *Azadirachta indica* and *Oscimum sanctum* were collected from the botanical garden of Govt. Holkar science college in poly bags and brought to lab and their botanical identity was established and *Allium sativum* was brought market and it was also identified at the department of botany, Govt. Holkar science college, Indore (M.P.). The collected material was washed, shade dried under room temperature ($27\pm 2^{\circ}\text{C}$) and *Azadirachta indica* and *Oscimum sanctum* was powdered using electric blender and the paste of *Allium sativum* was made which were further processed for phytochemical analysis.

Experimental plant product used for extract

Following plant products were used for extract preparation

Azadirachta indica- Leaves

Allium sativum - Bulbs

Ocimum sanctum- Leaves

Soxhlet extraction

The ordinary method of extraction was not efficient to yield good amount of active principle of the plant material. To extract more active principle from all the plant materials, Soxhlet extraction given by Sharma and Gupta (2009) was used. The plant extract were prepared in two solvent one was distilled water and second was ethanol.

Phytochemical screening

Phytochemical screening was done in order to detect the presence of bioactive constituents such as alkaloids, tannins, saponins, phenols, glycosides, flavonoids and glycosides using the methods described by Sofowora (1978), Trease and Evans (1989).

Experimental insect and rearing

Maize stem borer; *Chilo partellus* (Swinhoe) was selected for the present investigation. A series of surveys were undertaken for the collection of larvae in the maize fields. The sampling was conducted two times at the vegetative and reproductive stage (tasseling and soft dough stages). In each stage, evaluation was conducted, each maize plant was dissected and the larvae of the pests per plant were counted and placed in wide-mounted jars. Rearing of larvae was done in accordance with Koul et al. (2013).

Antifeedant Bioassays: Antifeedant effects were investigated using leaf disk choice bioassays (Sharma and Gupta, 2009) The feeding inhibition was calculated using the formula (Isman et al., 1990)

$$\text{Percent area consumed} = 100 - \frac{\text{Area of leaf fed in treatment}}{\text{Initial leaf area given for feeding}} \times 100$$

The data were corrected with respect to control using the Abbott's formula (Abbott, 1925) as under:-

$$\text{Corrected per cent antifeedant effect} = \frac{T - C}{100 - C} \times 100$$

Where, T = per cent area consumed in treatment
C = per cent area consumed in control

All data thus obtained was tabulated and graphically presented as per the required statistical methods. The data was analyzed by analysis of variance (ANOVA) using statistical software.

RESULTS

Phytochemical screening

In the present investigation phytochemical screening were done in order to detect the presence of bioactive constituents. The plant extracts (*Allium sativum*-bulb, *Azadirachta indica*- Leaf and *Ocimum sanctum*- Leaf) in different solvent (Aqueous and Ethanol) were screened for the presence of various bioactive phytochemical compounds. The analysis revealed the presence of alkaloids, glycosides, tannins, saponins, phenolics and

flavonoids. The results of phytochemical screening of different plants summarized in table-1.

The phytochemical screening of *Azadirachta indica* leaves showed the presence of phenolics, flavonoid and saponins, and absence of glycosides, steroids, tannins and

alkaloids in aqueous extract. In the ethanolic extract glycosides, phenolics, flavonoid, tannins, alkaloids and saponins are present and steroids absent.

The phytochemical screening of *Allium sativum*- bulb showed the presence of glycosides, steroids, phenolics, flavonoid, tannins, alkaloids and saponins in both aqueous as well as ethanolic extract.

The phytochemical screening of *Oscimum sanctum* leaves showed the presence of Phenolics, Saponins, Steroids, Tannins and Alkaloids and the absence of glycosides and Flavonoid in aqueous extract. In the ethanolic extract Alkaloids, Phenolics Saponins and Steroids are present and glycosides, Tannins and Flavonoid are absent. The results of phytochemical screening were summarized in table 1.

Table-1 Phytochemical screening of *Azadirachta indica* (Leaves), *Allium sativum* (Bulbs) and *Oscimum sanctum*(Leaves)

S.no.	Plant materials	Phytochemicals	Aqueous extract	Ethanolic extract
1.	<i>Azadirachta indica</i>	Glycosides		+
		Steroids		
		Phenolics	+	+
		Flavonoids	+	+
		Tannins		+
		Alkaloids	+	+
2.	<i>Allium sativum</i>	Saponin	+	+
		Glycosides	+	+
		Steroids	+	+
		Phenolics	+	+
		Flavonoids	+	+
		Tannins	+	+
3.	<i>Oscimum sanctum</i>	Alkaloids	+	+
		Saponin	+	+
		Glycosides		
		Steroids	+	+
		Phenolics	+	+
		Flavonoids		

Keys = (+) indicates presence and (-) indicates absence of the components.

Antifeedant effect

In the present investigation, antifeedant effect of different plant extract with different concentrations (2.0 % - 10.0 %) were assessed by comparing the averages of the leaf area consumed in the treated leaves and control leaves. Antifeedant activity of the plant extracts of *Allium sativum*-bulbs, *Azadirachta indica*-leaves and *Oscimum sativum*-leaves studied at different concentrations are summarized in table 2-6 and presented by figure 1-5.

Antifeedant activity of different plant extracts with different solvents was assessed based on antifeedant index. Higher antifeedant index indicates decreased rate of feeding. Data pertaining to the below tables clearly indicated that the maximum antifeedant activity was recorded in ethanolic extract of *Azadirachta indica*-leaves (80.33 %) at 10.0 % concentration followed by *Allium sativum*-bulbs (74.37 %) at 10.0 % concentration. The minimum antifeedant effect was showed by aqueous extract of *oscimum sanctum*-leaves (2.09 %) at 2.0 % concentration. Thus the above result reveals that as the

concentration of plant extracts increases, the antifeedant effect also increases. Therefore, in general the antifeedant effect of different concentrations, irrespective of the extracts decreased with decrease in concentration from 10 % to 2.0 %.

Table 1 Percent antifeedant activity of different plant extracts at 2.0 % concentration on larvae (fourth instars) of *Chilo partellus*.

Plant extracts	Aqueous extract				Ethanolic extract			
	12h	24h	48h	Mean±SD	12h	24h	48h	Mean±SD
<i>Allium sativum</i>	16.90	27.68	64.13	36.2±24.8	17.84	28.65	66.95	37.81±25.8
<i>Azadirachta indica</i>	30.36	40.75	69.74	46.9±20.9	31.79	42.17	73.98	49.31±22.0
<i>Oscimum sanctum</i>	2.09	15.15	31.40	16.2±14.7	3.19	14.60	53.15	23.64±26.2

Within the column, mean ± SD differ significantly P<0.05

Table 2 Percent antifeedant activity of different plant extracts at 4.0% concentration on larvae (fourth instars) of *Chilo partellus*

Plant extracts	Aqueous extract				Ethanolic extract			
	12h	24h	48h	Mean±SD	12h	24h	48h	Mean±SD
<i>Allium sativum</i>	18.81	29.68	66.63	37.37±25.1	19.62	30.62	69.14	39.79±26.0
<i>Azadirachta indica</i>	32.13	42.88	72.25	49.08±20.8	33.31	44.02	76.61	51.31±22.6
<i>Oscimum sanctum</i>	4.01	17.26	53.27	24.84±20.5	4.79	16.25	55.22	25.42±20.4

Within the column, mean ± SD differ significantly P<0.05

Table 3 Percent antifeedant activity of different plant extracts at 6.0% concentration on larvae (fourth instars) of *Chilo partellus*

Plant extracts	Aqueous extract				Ethanolic extract			
	12h	24h	48h	Mean±SD	12h	24h	48h	Mean±SD
<i>Allium sativum</i>	19.19	30.18	68.94	39.43±26.1	21.41	32.55	69.89	41.28±25.4
<i>Azadirachta indica</i>	33.88	44.27	74.64	50.93±21.2	35.17	46.01	76.97	52.71±21.7
<i>Oscimum sanctum</i>	5.77	17.65	55.71	26.37±26.0	6.60	18.20	57.70	27.5±26.7

Within the column, mean ± SD differ significantly P<0.05

Table 4 Percent antifeedant activity of different plant extracts at 8.0% concentration on larvae (fourth instars) of *Chilo partellus*.

Plant extracts	Aqueous extract				Ethanolic extract			
	12h	24h	48h	Mean±SD	12h	24h	48h	Mean±SD
<i>Allium sativum</i>	20.92	32.15	69.52	40.86±25.4	21.77	32.93	72.27	42.32±26.5
<i>Azadirachta indica</i>	35.62	46.27	75.37	52.42±20.6	36.95	47.90	79.07	54.64±21.9
<i>Oscimum sanctum</i>	7.20	19.53	57.77	28.16±26.4	8.45	20.10	59.96	29.50±27.0

Within the column, mean ± SD differ significantly P<0.05

Table 5 Percent antifeedant activity of different plant extracts at 10 % concentration on larvae (fourth instars) of *Chilo partellus*.

Plant extracts	Aqueous extract				Ethanolic extract			
	12h	24h	48h	Mean±SD	12h	24h	48h	Mean±SD
<i>Allium sativum</i>	22.35	33.36	71.54	42.41±25.8	23.53	34.63	74.37	44.17±26.7
<i>Azadirachta indica</i>	37.31	48.01	77.70	54.34±20.9	38.64	48.73	80.33	55.9±21.8
<i>Oscimum sanctum</i>	8.70	21.17	60.07	29.98±26.8	10.16	23.43	62.51	32.03±27.2

Within the column, mean ± SD differ significantly P<0.05

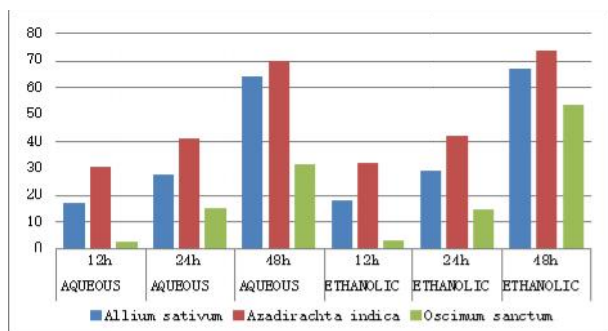


Figure 1 Graph showing antifeedant effect of *Azadirachta indica*-leaves, *Allium sativum*-bulbs and *Oscimum sanctum*-leaves on the larvae (fourth instar) of *Chilo partellus* at 2.0 %concentration.

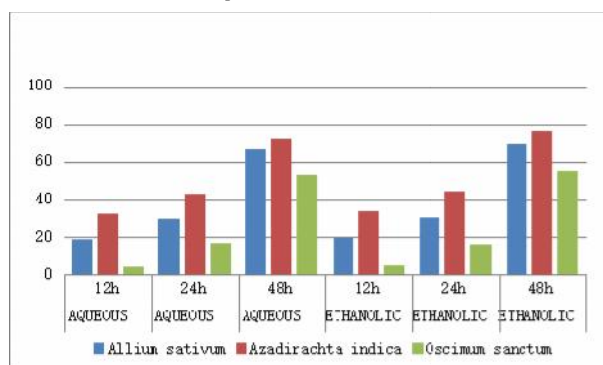


Figure 2 Graph showing antifeedant effect of *Azadirachta indica*-leaves, *Allium sativum*-bulbs and *Oscimum sanctum*-leaves on the larvae (fourth instar) of *Chilo partellus* at 4.0 %concentration.

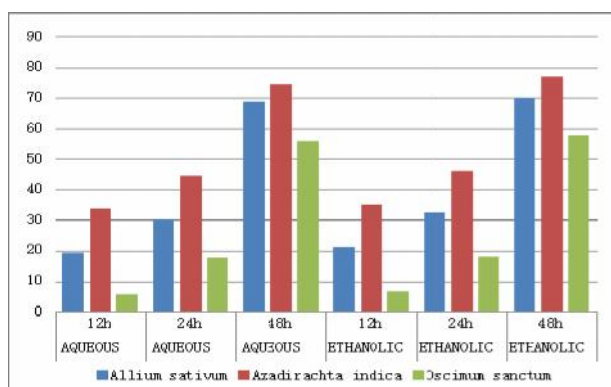


Figure 3 Graph showing antifeedant effect of *Azadirachta indica*-leaves, *Allium sativum*-bulbs and *Oscimum sanctum*-leaves on the larvae (fourth instar) of *Chilo partellus* at 6.0 %concentration.

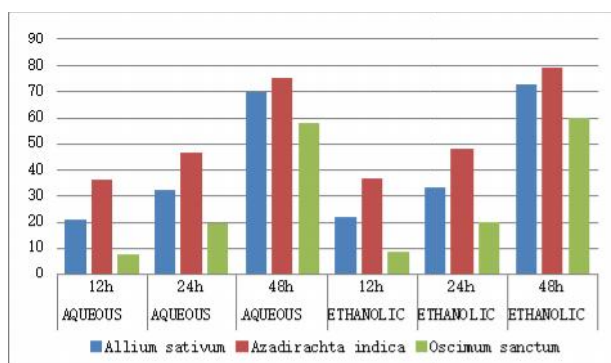


Figure 4 Graph showing antifeedant effect of *Azadirachta indica*-leaves, *Allium sativum*-bulbs and *Oscimum sanctum*-leaves on the larvae (fourth instar) of *Chilo partellus* at 8.0 %concentration.

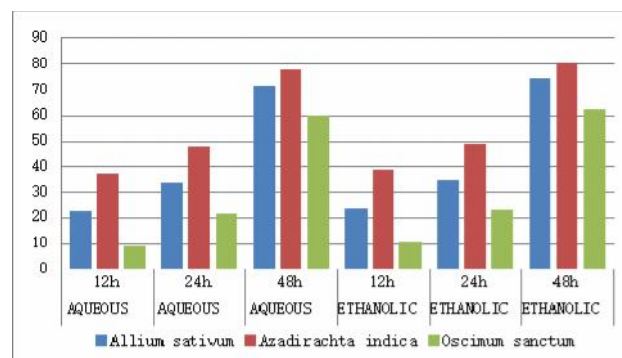


Figure 5 Graph showing antifeedant effect of *Azadirachta indica*-leaves, *Allium sativum*-bulbs and *Oscimum sanctum*-leaves on the larvae (fourth instar) of *Chilo partellus* at 10 %concentration.

DISCUSSION

A medicinal plant is any plant in which, one or more of its parts, contains substances that can be used for therapeutic purpose of which are precursors for the synthesis of useful drugs. The crude extracts or purified form of plant has been used as medicines and cosmetics (Sofowora, 1993). The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmoladun et al., 2007). In our present study the phytochemical investigation of *Allium sativum* (Bulbs), *Azadirachta indica* (leaves) and *oscimum sanctum* (leaves), indicates the presence of alkaloids, flavonoids, saponin, tannins and cardiac glycosides. This is in agreement with the work done by Idowu et al. (2008). These classes of compounds especially alkaloids, saponins, tannins and flavonoids are known to have curative activity against several pathogens (Usman et al., 2009).

Dutta (1993) also reported some compounds as an indication of the potential medicinal value of the plants in which they appear. The results of present study the phytochemical screening indicated the presence of phenolics, alkaloids, flavonoids, steroids, glycosides, saponins, and tannins. The result is in agreement with the work of Lawal et al. (2010) who reported that garlic extract revealed the presence of alkaloids, saponins, tannins, flavonoids, glycosides, cardiac glycosides, volatile oils and steroids. The result of present study of phytochemical analysis of *Azadirachta indica* (leaves) is also in agreement with Kraus et al. (1981). The result of *Oscimum sanctum* is also supported by the work of Rama et al. (2012).

Ethanollic plant extract against larvae of *Chilo partellus*, at different concentrations caused minimum 73.98 % and maximum 80.33 % protection to the foliage over control (Table 2-6 and figure 1-5). Extract of *Azadirachta indica* resulted in maximum protection to foliage over control at all the concentrations used followed by *Allium sativum* and than *oscimum sanctum* against *Chilo partellus* (fourth instars).

Many researchers like Saxena et al. (1981), Chapman, 1974 and Schoonhoven, 1982 were also in agreement with the present work on feeding deterrency and repellency of neem. They also worked for the antifeedant property of neem leaf

extract on various agricultural pests and found the highest antifeedant effect shown by neem therefore their work also supported present investigation. Banerji and Rembold (1982) and Dancewicz *et al.* (2011) also was in agreement that *Allium sativum* and *Oscimum sanctum* suppress the feeding behaviour of *Myzus persicae*. They also reported the feeding deterrent efficacy of *Allium sativum* extract against mustard saw fly *Athalia proxima*.

The present findings suggest that plant extracts of *Azadirachta indica*, *Allium sativum* and *Oscimum sanctum* contain biologically active compound which possesses a potentially vital antifeedant effect on *C. partellus*. Thus, these plant extracts offers significant promise for combating the threat posed by maize stem borer to farmers. The major thrust of this work is its adaptability for use by small scale farmers plagued by the challenge of not being able to afford conventional pesticides on the market.

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