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Insecticidal activity of *Dryopteris filix-mas* (Linn.) Schott ethanolic extract against *Corcyra cephalonica* Staint. (Lepidoptera: Pyralidae)

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ABSTRACT

Larvicidal and pupicidal effects of *Dryopteris filix-mas* root and rhizome's ethanolic extract were studied against the third instar larvae of *Corcyra cephalonica* (Staint.). *D. filix-mas* extract 0.20% (v/w), caused 100% larval mortality. The plant extracts reduce pupation per cent, pupal death and adult emergence indicating absolute toxicity to the pest. However, further detailed studies are essential under pots, controlled-field cage and field studies.

Key words: *Corcyra cephalonica*, ontogeny, *Dryopteris filix*-mas, toxicity

INTRODUCTION

The rice-moth, *Corcyra cephalonica* Staint. (Lepidoptera) is a serious pest of stored cereals and cereal commodities in India as well as in other tropical and subtropical regions of the world. Its larval stages cause appreciable loss to rice, sorghum, currants, grams, cocoa beans peanuts, cotton seed, linseed, raisins, chocolates, army biscuits, nutmeg and milled products (Atwal, 1976; Piltz, 1977).

The use of chemical agents to prevent or control insect infestations has been the main method of grain protection, since it is the simplest and most cost-effective means of dealing with stored products pest (Hidalgo *et al*., 1998). However, insecticides have serious drawbacks such as pest resurgence and resistance, lethal effects on non-target organisms, the risk of user's contamination, food residues, and environmental pollution (Tapondjou *et al.*, 2002). In addition, the precaution necessary to work with traditional chemical insecticides (Fields *et al*., 2001), and the poor storage facilities of traditional farmers in developing countries, which are unsuitable for effective conventional chemical control (Tapondjou *et al*., 2002), emphasize the necessity of new and effective methods for the insect pest control of stored products.

Thus, there is an urgent need to develop safe alternatives to conventional insecticides and fumigants for the protection of grain products against insect infestations. Higher plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control (Jbilou *et al*., 2006). Plant materials with insecticidal properties have been used traditionally for generation throughout the world (Belmain *et al*., 2001). Hill (1990) reports that, 2000 species of plants, which produce chemical substances able to act against, are known. The substances can have poisonous and repellent effect, and can work as phagos restrainer, ovicide and can affect the insect hormonal system. Several plants like *Annona squamosa*, *Tephrosia purpurea* and *Acorus calamus* are reported for their toxic properties against insect pests (Atwal, 1986; Jadhav, 2009).

Different type of plant preparations such as powders, solvent extracts, essential oil and whole plants are being investigated for their insecticidal activity including their action as fumigants, repellents, antifeedants, anti-oviposition and insect growth regulators (Isman, 2000; Weaver and Subramanyam, 2000; Koul, 2004; Mordue, 2004; Erturk, 2004; Negahban and Moharramipour, 2007). Considerable efforts have been focused on plant derived materials, potentially useful as commercial insecticides. Toxic effects of plant products on some pests have been studied by many researchers (Essien, 2004; Erturk *et al*., 2004; Koona and Dorn, 2005). Since these plant materials with insecticidal properties are often active against a limited number of species, are often biodegradable to non-toxic products and are potentially suitable for use in integrated pest management, they could lead to the development of new classes of safer insect control agents (Kim *et al*., 2003).

Earlier findings reveal that the rhizome and young shoots (fiddleheads) of the male fern (*Dryopteris filix*-mas) have deworming properties that have long been recognized in Europe against tapeworms (Taenia). The ferns are effective in arresting embryonic development in insects. The extracts of pteridophytes have toxic effects on *Spodoptera littura* and

Helicoverpa armigera. Filicin, isolated from the rhizomes of *Dryopteris filix*-mas, is a potential insecticide (Mannan *et al*., 2008). Aquatic extract of root and rhizomes of *D. filix-mas* has been found to be effective against ontogeny (Shukla and Tiwari, 2011) as well as larval haemolymph and fat body biochemistry of this pest (Shukla, 2010). Diethyl ether and acetone extracts of *D. filix-mas* root and rhizome have been observed to be not effective against the ontogeny of this pest (Shukla, 2010).

The objective of the present work was to investigate the insecticidal effects of *D. filix-mas* root and rhizome's ethanolic extract to control the rice-moth, *C. cephalonica*. Hence as an objective of such programme the present work for the first time, has been designed and conducted to investigate the effect of *D. filix-mas* root and rhizomes powders against the ontogeny of rice-moth, *C. cephalonica*. Such knowledge may be regarded as one of the objective criteria permitting an assessment of effectiveness of botanical control measures against *C. cephalonica* in particular and lepidopterous pests in general.

MATERIALS AND METHODS

D. filix-mas plants were collected from adjacent areas of Gorakhpur and neighbour districts of U.P. Their root and rhizomes were separated from the plant body, properly washed with fresh tap water, cut into small pieces, cooked in boiling water for more than an hour to destroy the thiaminase, dried in sun light for six to seven days, pulverized in a mortar and pestle and then it was ground in an electric grinder. The powder so obtained was then extracted in ethanol in a sox let apparatus. The extracted substance was used for the experiment.

From the laboratory maintained culture on ground jowar mixed with 5% (w/w) yeast powder, newly emerged males and females were transferred to oviposition glass chambers (35 mm diameter, 200 mm height). Eggs laid by the females were collected and then placed in glass chambers (consisting of 250 ml beakers) for hatching. Freshly hatched larvae of *C. cephalonica* were allowed to feed on a normal dietary medium mixed with 5% (w/w) yeast powder kept inside 250 ml beakers for exactly 15 days. On the 16th day of larval hatching, 25 third instar larvae were transferred to each similar rearing chambers (250 ml beakers) containing 50 gms of dietary medium mixed and treated separately with 11 dose levels of *D. filix-mas* ethanol extract (0.01, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18 and 0.20%) using 5 replications of each treated as well as normal dietary media as control. For the preparation of different dose levels of *D. filix-mas* in dietary media, a stock solution of *D. filix-mas* ethanolic extract was prepared by dissolving it in a required volume of ethanol and then adjusted via serial dilution to achieve its different required concentrations (a.i.) v/v in ethanol. Required quantities of different concentrations of ethanolic extract of *D. filix*-mas were thoroughly mixed with the required amount of food separately and then air dried at room temperature to eliminate the excess of ethanol completely. For control purposes, the normal food was mixed with a definite volume of ethanol similar to that of *D. filix*-mas extract mixed with dietary media. On completion of the life-cycle, the number of adults emerged and dead pupae were recorded to calculate percent pupation and percent larval mortality. Experiments have been replicated 5 times and their values have been expressed as the mean ± S.D. of five replicates. Straight line regression equation was applied between different dose levels of *D. filix*-mas extract and their corresponding per cent larval death / per cent pupation / per cent pupal death and percent adult emergence to observe the significant correlation (Sokal and Rohlf, 1969). The amount of insecticide consumed by larvae were calculated as µg / larva at each dose level of *D. filix*-mas ethanol extract. LD_{50} values (µg/larva), 95 % confidence limits (lower and upper confidence limits) of LD_{50}, slope values, g values and heterogeneity of ethanolic extract of *D. filix-mas* root and rhizome, were calculated by Polo Plus, Probit and Logit Analysis, Version: 2.0, LeOra Software based on probit analysis (Finney, 1959).

RESULTS

Results presented in Table 1 reveal that a significant larval mortality was obtained with the increase of *D. filix-mas* root and rhizome's ethanolic extract dose levels. At 0.01% dose level of this ethanolic extract larval mortality was only 4 ± 2.82 % while 100 % larval mortality was observed at its 0.20 % dose level. With the increase of this ethanolic dose levels a significant reduction in pupation and a significant enhancement in pupal death occur. 96 ± 2.82 % pupation was recorded at 0.01% dose level which decreased to 13 ± 1.80 at 0.18% dose level of *D. filix-mas* ethanolic extract. During the same observation period, 2.32 ± 3.60 % pupal deaths was recorded at 0.04%, which increased to 100 % at 0.18 %. A significant reduction in adult emergence was recorded following exposure of increased dose levels of *D. filix-mas* ethanolic extract. At 0.01 % dose level of this botanical extract 96 ± 2.82 % adult emergence was recorded that decreased to 13 ± 1.80 % at its 0.16 % dose level. Per cent larval death ($y = -2.0327 + 506.659x$; $r = 0.99$, $P < 0.001$) and pupal death ($y = -12.866 + 365.97x$; $r = 0.76$, $P > 0.05$) were positively correlated, whereas per cent pupation ($y = 101.920 – 504.527x$; $r = -0.99$, $P < 0.001$) and emergence ($y = 102.60 – 560.273x$; $r = -0.99$, $P < 0.001$) were negatively correlated to the plant extract concentrations. It deserves mention that in control dietary media since food is richly supplied with yeast powder so no

Table 1. Toxicity of *Dryopteris filix*-mas root and rhizome ethanolic extract against the ontogeny of rice moth, *C. cephalonica*.

% *Dryopteris filix*-mas root and rhizome ethanolic extract concentrations in food enriched with 5% (w/w) yeast powder	Percent* larval death	Percent* pupation	Percent* pupal death	Percent* adult emergence	Acute toxicity to the pest
Control	0	100	0	100	
0.01	4 ± 2.82	96 ± 2.82	0	96 ± 2.82	Non toxic
0.02	8 ± 2.82	92 ± 2.82	0	92 ± 2.82	Non toxic
0.04	14 ± 2.82	86 ± 1.80	2.32 ± 3.60	84 ± 2.82	Poorly toxic
0.06	25 ± 1.80	75 ± 1.80	4.00 ±1.80	72 ± 2.82	Moderately toxic
0.08	40 ± 2.82	60 ± 2.82	5.00 ± 2.23	57 ± 1.80	Moderately severe
0.10	51 ± 1.80	49 ± 1.80	10.20 ± 4.38	44 ± 4.00	Moderately severe
0.12	62 ± 2.23	38 ± 2.23	13.15 ± 1.80	33 ± 1.80	Severely severe
0.14	68 ± 2.82	32 ± 2.28	18.75 ± 2.23	26 ± 4.58	Severely toxic
0.16	79 ± 1.80	21 ± 1.80	38.09 ± 2.23	13 ± 1.80	Severely toxic
0.18	87 ± 4.38	13 ± 4.38	100	______	Extremely toxic
0.20	100	______	______	______	Extremely toxic

* Values have been expressed as the mean ± S.D. of five replicates.

mortality is found in control and hence Abbott's formula is not applicable.

DISCUSSION

The present investigation reveals that different dose levels of ethanolic extract of *D. filix-mas* root and rhizome's exerted a depressive effect on the life cycle stages of *C. cephalonica*. The toxicity of this botanical extract increases with the increase in its sdose levels on each developmental stage. i.e. larva, pupa and adults. On the basis of % larval death, % pupation, % pupal death and % adult emergence, at different dose levels of *D. filix-mas* root and rhizome's ethanolic extract it is possible to categorise the relative effectiveness of their dose levels (Fitzpatrick and Dowell, 1981). *D. filix-mas* ethanolic extract at 0.20 % is considered to be extremely toxic to the pest, as no pupation occurs at this dose level indicating 100% larval mortality. At 0.18 % dose level of this ethanolic extract, pupation takes place but there is no emergence of any single adult indicating 100% pupal death. This dose level is also regarded to be extremely toxic to the pest. At dose levels 0.16, 0.14 and 0.12 % of this ethanolic extract the average emergence is 13, 26 and 33%, respectively. These dose levels are considered as severely toxic to the pest. A moderately severe toxicity is accounted to the dose levels of 0.10 and 0.08 % of this extract as the average emergence of these dose levels were 44 and 57 %, respectively. At 0.06 % dose level of *D. filix-mas* ethanolic extract the average emergence is 72 %. This dose level is considered as moderately toxic to the pest.

The average emergence, at 0.04% of this ethanolic extract is 84 %. This dose level may be regarded as poorly toxic to the pest. At 0.02 and 0.01% dose levels of *D. filix-mas* ethanolic extract, the average emergence is 92 and 96 %, respectively which may be considered to be non toxic to the pest.

Earlier findings reveal that 0.16% aqueous extract of *D. filix-mas* root and rhizome caused 100% larval mortality of *C. cephalonica* (Shukla and Tiwari, 2011) while their diethyl ether and acetone extracts reveal negligible toxicity against the ontogeny of this pest (Shukla, 2010). Investigations have revealed that natural plant products extracted in different organic solvents show different degree of toxicity to different insect population. Acetone extracts from fresh and stored leaves of *A. squamosa* were found to be toxic to *C. maculatus* whereas its ethanolic extracts were not active (Dharmasena *et al*., 2001). Contrary to this investigation, in the present study, acetonic extracts of *D. filix-mas* was observed to be not effective against the ontogeny of *C. cephalonica*, where as its ethanolic extracts was found to be effective. Similarly, petroleum ether extracts of seed of *A. squamosa* was found to be effective against *S. litura* (Boreddi and Chitra, 2001) but contrary to this diethyl ether extract of *D. filix*-mas was observed to be not effective against the ontogeny of rice-moth, in the present investigation (Shukla, 2010).

Various plant products have been reported to control the stored cereal pests. The extracts of *Verbascum cheiranthifolium* and *V. speciosum* either suppressed oviposition or killed the larvae of *S. oryzae* hatching from eggs laid in the medium culture. These results suggest that there may be different compound in extracts possessing different bioactivities (Khoshnoud *et al*., 2008). Saxena *et al*. (1992); Bhattacharya (1993); Senguttuvan *et al*. (1995); Saxena *et al*. (1996); Sahayaraj *et al*. (2007) and Jagajothi and Martin (2010) studied the effect of different plant extracts on insect pests and found several to be toxic to different insects.

Studies regarding mode of action of botanicals have revealed that active ingredients present in the insecticidal plants inhibit some of the enzyme systems and subsequent mortality (Ryan and Byrne, 1988; Chaterjee and Prakashi, 1995; Kuroyanagi *et al*., 1999; Padmavati and Reddi, 1999). Active ingredients of leaves of Calotropis and Ipomea have been reported to inhibit some of the enzyme systems and subsequent mortality (Kuroyanagi *et al*., 1999; Padmavati and Reddi, 1999). Active ingredients of Ocimum has also been known to act as a reversible competitive inhibitor of acetyl cholinesterase (Ryan and Byrne, 1988) like azadirachtin, abundantly present in the neem seeds that have been shown to inhibit the release of prothoracicotropic hormones and allatropins (Banken and Stark, 1997), thereby affecting metamorphosis in insect (Schmutterer and Rembold, 1995) is well documented. It also alters insect behaviour because of its anti-feedant and repellent action, and it modifies insect development by inhibiting the release of prothoracicotropic hormones and allatotropins [Mordue (Luntz) and Blackwell (1993) and Williams and Mansingh (1996)].

The findings of our study are in accordance with Saxena *et al*. (1992); Bhattacharya (1993); Senguttuvan *et al*. (1995); Schmutterer (1995); Mostafa *et al*. (1996); Saxena *et al*. (1996); Musabyimana *et al*. (2001); Tinzaara *et al*. (2006); Sahayaraj *et al*. (2007); Khoshnoud *et al*. (2008); Jagajothi and Martin (2010) reported toxicity similar to the findings in the present investigation.

Today, the environment safety of an insecticide is considered to be of paramount importance. The world flora has a variety of plant species and in order to increase the number of plants used for pest control, more studies should be carried out. Thus, a variety of effective substance found in different plant species could be discovered. In addition, cheaper pesticides can be obtained and environmental pollution will gradually decrease.

From the present investigation, it may be concluded that 0.18% and 0.20% dose levels of *D. filix-mas* root and rhizome's ethanolic extract are efficient for the effective control of *C. cephalonica* in particular and lepidopterous pests in general.

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