Insecticidal activities of neem gold on banana rhizome weevil (BRW), *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae)

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ABSTRACT

The banana weevil (BRW), *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is the most devastating pest of banana in most production areas, which limits production and productivity of bananas throughout the world. Numerous chemical insecticides are available commercially to manage this pest. However, it was more expensive and undesirable for the management of this pest. In this study, the effect of commercially available neem-based botanical insecticide neem gold on BRW was studied under laboratory condition. The weevils were separately maintained up to 96 hours on neem gold treated banana rhizomes. The total and differential haemocytes count; total body protein and fat body protein profile were performed using SDS-PAGE. 33.33% of *C. sordidus* were settled on neem-gold and significantly ($P<0.05$) caused minimum mortality (6.7%) whereas 66.6% weevils preferred the untreated pseudostem. However, treatment reduced both total haemocyte count (40%) and various haemocytes level. The total body protein and fat body protein analysis shows that 4 KDa to 163 KDa molecular weight and 144 to 7 KDa molecular weight polypeptides respectively are present. Consumption of neem gold impregnated banana pseudostem reduced the total body protein polypeptide to 8 KDa whereas the fat body polypeptide increased from 161 to 8 KDa. These results suggested that neem gold could be used as insecticide against *C. sordidus.*

Keywords: *Cosmopolites sordidus,* crop pest, neem gold, botanicals, banana, body and fat body protein

INTRODUCTION

Bananas (*Musa* spp) are the major food crop globally under grown and consumed in more than 100 countries throughout the tropics and subtropics (Tiwari *et al.,* 2006). It is a perennial plant that produces new plants by suckering. Plant emerging from the same corm comprises a banana mat. Each plant produces a single bunch (Masanza *et al.,* 2005). In developing countries they are the most important food crop after rice, wheat and maize (INIBAP, 2000). Worldwide over 1000 banana cultivators or land races are recognized (Helslop – Harrison and Schwarachar, 2007) for banana cultivation. India is the largest producer of banana in the world. Among the insect pest of banana, rhizome weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is one of the major constraints to banana production especially in small scale farming system in all the banana growing regions of the world (Gold *et al.,* 2001, Fogain *et al.,* 2002). It is known to interfere with root initiation, kill existing root, limit nutrient uptake, reduce plant vigor, delay flowering and increase susceptibility to other pests and diseases (Budenberg *et al*., 1993; Gold and Messian., 2000; Gold *et al.,* 2004). *C. sordidus* is native to Malaysia and Indonesia but has spread nearly to all the banana growing areas of the world (Gold *et al.,* 2001).

Rukazambuga (1998), Gold and Messiaen (2000) estimated, about 44% of yield losses due to banana weevil in the third ratoon cycle. In East Africa, *C. sordidus* has been found to cause upto 100% yield losses (Gold *et al.,* 2004) among the highland cooking bananas (Genome group EA-AAA) and in severe weevil infestations, crop losses up to 100% have been reported (Sengooba., 1986 and Andrew Kiggundu., 2003). The borers cause yield reduction by sucker establishment in newly planted crops (McIntyre *et al.,* 2001). Banana weevil management is currently based on the application of cultural practices (Andrew Kiggundu *et al.,* 2003), chemical insecticides (Collins *et al*., 1991). Since synthetic insecticides cause too many problems (Nethi Somasekhar, 2007), biopesticides, especially naturally occurring pesticides have a prominent role in the development of next generation botanical insecticides for a safe, clean and healthy environment (Sahayaraj *et al.,* 2008). Botanicals have variously been reported as possessing insecticidal properties against a wide range

of insects particularly soil borne insects though sparse (Inyang and Emosainue, 2005). Among the botanicals, extracts from neem tree (*Azadiracta indica* Juss.) and commercially viable products have been most extensively studied and used in the last decade (Schmutterer, 1990; Musabyiamana *et al*., 2001 and Tinzaara *et al.*, 2006). Neem gold is one among the commercially available neem based botanicals containing azadirachtin (Sharma *et al*., 2003).

The present study was undertaken to find out the olfactory response and insecticidal activity of banana rhizome weevil on the neem gold and also to evaluate its qualitative changes in the proteins of total body and fat body, total and differential haemocyte population of *C. sordidus*.

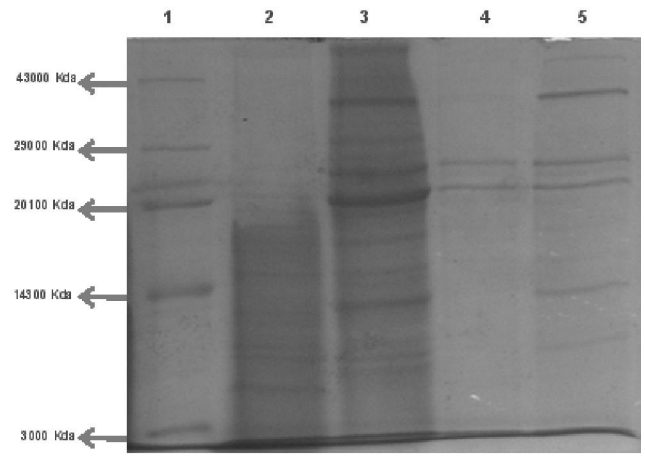

Figure 1. SDS-PAGE protein patterns of total body and fat body profile of *Cosmopolites sordidus*.

MATERIALS AND METHODS

Insects

Adult *C. sordidus* were collected from the banana field in and around Kanyakumari district, Tamil Nadu and were maintained in the laboratory at 28 ± 2°C and 60 ± 10 % RH at 8: 16 LD photoperiod in plastic containers (10 cm diameter and 20 cm depth) with holes of 1mm diameter for aeration. The fresh pieces of rhizome and pseudostem were provided for its maintenance. Fed and unfed pseudo stems were changed once in three days. Laboratory maintained adult weevils were used for the experiment.

Olfactory Response

Bioassay was conducted under ambient laboratory conditions as mentioned above. The olfaction activity of the neem gold against the weevil *C. sordidus* was evaluated using two way olfactometer as described by Sahayaraj (2008). In brief, the olfactometer consists of release chamber at the centre and two side arms (one chamber for control and the other for the test sample). Commercially available neem gold (Southern Petrochemical Industries Corporation Limited) was purchased from local shop, 1% of this neem gold was prepared using distilled water by adding 0.05% of Teepol as adjuvant and kept separately in sealed glass jars before use. Pseudostem pieces were (3 cm x 2 cm) dipped for about five to ten minutes in the prepared neem gold solution and air dried for five minutes. The control pseudostems were dipped in distilled water alone. The treated pieces were placed at one chamber and the other chamber was provided with untreated pseudostem and closed with a fine cada cloth. Twelve hours pre-starved ten rhizome weevils were released at the centre of the olfactometer and their preference was observed after 1, 3, 5, 7, 9, 18 and 24 hours continuously. The experiment was replicated six times with different insects. The weevils preferred either fresh pseudostem or decayed pseudostem or neither. If the weevil chose neither of the chambers then it was considered that weevil made no choice. The number of weevils found on the treated and untreated pseudostem pieces was checked after 1, 3, 5, 7, 9, 18 and 24 hours continuously. From the observation recorded the Access proportion Index (API) was performed using the following formula Access Proportion Index = NS-NC/ NS+NC, Where NS = Number of insects choosing the sample side and NC = Number of insects choosing the control side. After 24 hours, the weevils settled in neem gold treated pseudostem was collected and reared with the same food up to 96 hours in separate plastic containers (500 ml capacity). Every 24 hours the pseudostems were replaced with fresh neem gold treated stem. After 4 days, live weevils were fed on fresh untreated pseudostem for a week. Live insects were used for the haemocyte and protein profile studies. Mortality was also recorded during the period.

Total (THC) and Differential Haemocyte Count (DHC)

The fore legs of *C. sorididuss* were amputated using fine sterilized scissors and the haemolymph was drawn using capillary tube (1mm diameter) and it was transferred to an eppendorf tube (1ml) containing anticoagulant buffer. The haemolymph was drawn into the WBC pipette (Naubauer's Haemocytometer) upto 0.5 marking. Then it was diluted with an acidified physiological saline (NaCl – 4.65g; KCl – 0.5 g; $CaCl_2$ – 0.11g; Gentian violet – 0.005g and acetic acid – 0.125ml and made up to 100ml using distilled water). The diluted haemolymph was drawn into the pipette upto mark 1.1 giving 20 times dilution. The inner surface of the WBC pipette was rinsed several times with diluting fluid before drawing the haemolymph. The cells in all the four 1mm square were counted and expressed in cell/mm^3

(Jones, 1962). For differential haemocyte count, oozed out haemolymph was spread out on clean micro slide and a neat thin clear film smear was made using a micro cover slip. It was then fixed with 0.1ml of 100% methanol and stained with 4% Giemsa stain and allowed to air dry for a few minutes. The preparation was observed under the Phase contrast microscope (Olympus CX 41). Two hundred cells were counted randomly per slide under oil immersion and phase contrast. The observed haemocytes were identified as per Tembhare (2000).

Protein Profile

After the haemolymph collection the insects were dissected in insect ringer's solution (Sodium chloride – 0.65g, Sodium carbonate – 0.02g, Calcium chloride – 0.03g, Potassium chloride – 0.025g and 100ml of distilled water), fat body was removed and washed thoroughly in the same. Five treated insects were homogenized in a homogenizer in 1ml of phosphate buffer (pH 7.2), centrifuged at 7,000 rpm for 10 minutes at 4^{o}C. From the treated insects, fat body was removed and prepared as mentioned above. The supernatant was stored at -10^{0}C and used as loading sample. SDS-PAGE (12% separating gel) was performed according to Laemmli (1970) procedure. Each well is loaded with 35 µl of sample along with a constant current of 50 volts for stacking and 100 volts for running gel for 3-4 hours. Gel was stained overnight in Commassie brilliant blue R. 250, then distained and photographed using gel documentation system (Biotech, India).

RESULTS AND DISCUSSION

Currently *C. sordidus* management mainly makes use of various practices like crop sanitation (Masanza *et al.*, 2005; 2006), pheromone trapping (Reddy *et al.*, 2009) and entomopathogens (Akello *et al.*, 2008) because of the threatened effects posed by chemical pesticides. In the present study we tried a neem-based botanical insecticide neem gold against *C. sordidus*. The result of olfactory response indicated that neem gold showed 33.3 per cent (API = -0.633) of weevils preferred neem gold at 24 hours observation (Table 1). At the same time neem gold signifi cantly ($P < 0.05$) caused minimum mortality (6.7%). However, it had an impact on haemocyte count and protein profile of the weevil. Previous reports also suggested that plant extracts such as neem, castor oil have limited insecticidal activity but may affect the reproductive behavior (Mostafa *et al.*, 1996; Musabyimana *et al.*, 2001). Musabyimana *et al.* (2000) reported that powdered neem seed and cake significantly reduced the infestation by *C. sordidus*.

Insects possess an open circulatory system which contains various types of haemocytes, the mesodermal cells which perform several physiological functions, including protection from pathogens (Sharma *et al*., 2003). As a new trend, researchers are now trying to use the haemolymph as a medium for controlling insect pests because, the changes occurring in haemolymph are expected to get transferred to other portions of the body and directly or indirectly affect the insects adversely (Bharti Prakash *et al.*, 2007). Botanical insecticide neem gold had a significant effect on the haemocyte profile of weevils. In control category, the total haemocyte population of the banana weevil was 3360 cells per mm^3 and 43 percent population was reduced in the neem gold treated weevils (Table 2). Sharma *et al*. (2003) reported that neem gold was significantly reducing the total haemocyte count of *Spodoptera litura* (Fab.). There are five types of haemocytes (Granulocytes, Prohaemocytes, Plasmatocytes, Cystocytes and Oenocytes) observed in the haemolymph of *C. sordidus*. Previously Mohammed AI-Khalifa and Mohammed Siddiqu, (1999) reported that the Red palm weevil *Rhynchephorus ferruginius* (Oliver)

Table 1. Settling response of *Cosmopolites sordidus* towards the neem gold treated rhizome.

Treatments	Hours after introduction						
	1h	3hrs	5hrs	7hrs	9hrs	18hrs	24hrs
Control	93.33	93.33	90.00	90.00	90.00	86.67	66.70
Neem gold	6.67	6.67	10.00	10.00	10.00	13.33	33.33
API	-0.8666	-0.866	-0.8	-0.8	-0.8	-0.733	-0.633

API – Access Proportion Index

Table 2. Effect of neem gold on total haemocyte count (cell/mm^3) and differential haemocyte (in %) count of banana rhizome weevil *Cosmopolites sordidus*

Treatments	Differential haemocyte count					Total haemocytes
	Granulocytes	Prohaemocytes	Plasmatocytes	Cystocytes	Oenocytes	
Control	39.38	19.69	08.59	22.36	09.97	3360
Neem gold	25.66	12.83	15.74	29.13	16.54	1440

Table 3. Effect of neem gold on the protein profile of banana rhizome weevil, *Cosmopolites sordidus* (Molecular weight in KDa)

Band Number	Total body protein		Fat body Protein	
	Control	Treatment	Control	Treatment
01	163277	164301	144509	161571
02	113628	155258	116528	145021
03	106633	152528	104585	116016
04	102197	142633	75239	105780
05	95543	133931	36509	100320
06	84794	125912	7675	89230
07	80528	118405		76434
08	69780	112604		61419
09	53571	101002		46746
10	49647	94348		38898
11	39239	82917		8358
12	34121	70633		
13	22690	65344		
14	4434	55960		
15		41628		
16		34121		
17		29855		
18		13135		
19		8017		

consists of six types of haemocytes (Prohaemocytes, plasmatocytes, Granular haemocytes, Cystocytes, Oenocytoids and adiphohaemocytes).

Proteins are the major biological factor that plays an important role in insect growth, development and various physiological processes. Figure 1 illustrates the SDS-PAGE protein patterns of the total body (lane 2 and 3) and fat body (lane 4 and 5) profile of *C. sordidus*. Total body protein profile of the weevils showed 14 bands in control. It was increased upto 19 bands in the neem gold treated category with the molecular weight ranging from 164 to 8 KDa. There were five new proteins that appeared in the treatment. The molecular weight of the polypeptides ranging from 4KDa to 163KDa in control. This result suggested that the neem-gold altered the normal physiological activity and induced the appearance of new polypeptides.

The protein patterns of the fat body shows a dramatic increase in the number of protein bands when compared to control. There are 6 bands (lane 4) present in control category; it was increased up to 11 bands (lane 5). The molecular weight of the fat body polypeptides ranged from 7KDa to 144KDa in untreated weevils but 8 to 161KDa in the neem gold treated weevils. This result also indicated that the consumption of neem gold induced the physiological changes in the fat body of the rhizome weevil *C. sordidus*. The results of both body and fat body suggested that the neem gold had the impact on protein profile of *C. sordidus*. From our investigation we concluded that neem gold could be used as insecticides for the management of banana rhizome weevil *Cosmopolites sordidus*.

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