



Available Online at EScience Press

## Plant Protection

 ISSN: 2617-1287 (Online), 2617-1279 (Print)  
<http://esciencepress.net/journals/PP>

### EFFICACY OF NEW CHEMISTRY INSECTICIDES ON THE HAEMOCYTES OF MAIZE STEM BORER, *CHILO PARTELLUS*, UNDER LABORATORY CONDITIONS

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#### ARTICLE INFO

##### Article history

 Received: 29<sup>th</sup> January, 2020

 Revised: 12<sup>th</sup> August, 2020

 Accepted: 16<sup>th</sup> August, 2020

##### Keywords

*Chilo partellus*

Haemocytes

Belt

Match

Radiant

Chlorfenpyr

#### ABSTRACT

In the present study, four new chemistry insecticides (Radiant, Chlorfenapyr, Belt and Match) were used to evaluate the haemocytological activity of *Chilo partellus*. LC<sub>50</sub> and LC<sub>60</sub> values of each insecticide were calculated by bioassay test. After the application of Radiant insecticide, the total haemocytes count immediately increased (78548.5 cells/mm<sup>3</sup>) as compared to normal (71255 cells/mm<sup>3</sup>), however, after 30 minutes decreased to (71694 cells/mm<sup>3</sup>) and after 60 minutes of interval, the total haemocytes count again increased (80215 cell/mm<sup>3</sup>). Percent plasmotocytes increased from untreated to 26%, while the spherulocytes, granulocytes, prohaemocytes and cystocytes also fluctuated from the untreated as 26%, 6.75%, 33% and 3.75% respectively. After the application of Chlorfenpyr insecticide, the total Haemocytes count immediately increased (80920.75 cells/mm<sup>3</sup>) as compared to normal (71255 cells/mm<sup>3</sup>), however after 30 minutes decreased to (71155 cells/mm<sup>3</sup>) and after 60 minutes, the total haemocytes count again increased (84100 cell/mm<sup>3</sup>). Percent plasmotocytes increased from untreated to 36%, while the spherulocytes, granulocytes, prohaemocytes and cystocytes also fluctuated from the untreated to 23.5%, 5.5%, 29% and 1.5% respectively. After the application of Belt insecticide, the total haemocytes count immediately increased (87200 cells/mm<sup>3</sup>) as compared to normal (71255 cells/mm<sup>3</sup>) and after 30 minutes decreased (71270 cells/mm<sup>3</sup>) and after 60 minutes, the total haemocytes count again increased (89640 cell/mm<sup>3</sup>). Percent plasmotocytes increased from untreated to 36%, while the spherulocytes, granulocytes, prohaemocytes and cystocytes also fluctuated from the untreated to 32.5%, 8.5%, 17.25% and 4.75% respectively. After the application of Match insecticide, the total haemocytes count immediately increased (87500 cells/mm<sup>3</sup>) as compared to normal (72155 cells/mm<sup>3</sup>) while after 30 minutes decreased (72190 cells/mm<sup>3</sup>) and after 60 minutes, the total haemocytes count again increased (99800 cell/mm<sup>3</sup>). Percent plasmotocytes increased from untreated to 33%, while the spherulocytes, granulocytes, prohaemocytes and cystocytes also fluctuated from the untreated to 31%, 14%, 23% and 4% respectively.

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## INTRODUCTION

Maize (*Zea mays*) is one of the most important cereal crops and ranks 3<sup>rd</sup> in the world cereal production after wheat and rice (Chouraddi et al., 2016; Manjunath and Mallapur, 2017). Maize is widely used as staple food, animal feed and fodder in the world. The grains of maize crop are widely used as raw materials for many textile and industrial products and have high nutritional values. In Pakistan, this crop is the major crop of spring and autumn seasons grown over 0.9 million hectares every year. The yield of maize crop in Pakistan is very low as compared with other maize grown countries (Anonymous, 2017). Various factors are responsible for this low yield and one of them is the maize stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) which is very destructive insect pest of many countries from Africa and Asia including Pakistan (Nabeel et al., 2018). Among the insect pests, *C. partellus*, has the potential to cause upto 75% crop losses (Kumar and Alam, 2017; Songa et al., 2001).

This insect pest has great potential to cause economic damage to the maize crop and has the ability to cause 100% damage at seedling stage of maize crop (Ali and Issa, 2019). At the milking stage, this pest can decrease the grain yield upto 81% (Kumar et al., 2018). The main reasons for this high damage to the maize crop is the lack of proper resistance varieties, which perform significant role in controlling and feeding behavior of the pest (Murenga et al., 2018).

The female lays eggs on the tender leaves and newly hatched larvae feed on these leaves and later on the larvae bores into the midrib, stalk and feed inside maize stem. This feeding behavior prevents it from the attack of natural enemies and insecticides (Siddalingappa et al., 2010). Various control measures have been used to manage this pest including cultural control (Dhaliwal et al., 2017) and biological control methods (Saeed et al., 2014).

Chemical control is a quick way to control insect pests (Kumar and Alam, 2017; Malik et al., 2015). These chemical insecticides have various types of formulations like granular formulations. Insecticides mostly recommended as soil and/or whorl application after 25 and 45 days of sowing of maize crop (Khan and Yamin, 2004). Moreover, foliar application of insecticides is effective as compared to granular formulation (Sidar et al., 2017). The insect pest can also be controlled by the whorl application of insecticides (granular and foliar) in liquid

formulations (Gunewardena and Madugalla, 2012).

All species of *C. partellus* have well developed cellular and humeral resistance structure in insect body that prevents them from the chemical, harsh conditions and disease causing agents. Insect immune systems eradicate the invaded tissues when identified (Fatima et al., 2013). Insect cellular immune structure consists of haemocytes which travel in blood of insect and usually interact with the tissues of insect body, whereas humeral immune reaction comprises of soluble molecules production (Strand, 2008).

Insect haemocytes play a significant role in the physical structure of the organisms. Haemocyte structure and function primarily have been considered in genetic or physical structure of insect models as well as the fruit fly (Ulvila et al., 2011). Haemocytes are generated from the embryonic medium mesodermal tissues and are separated into various types through embryogenesis (Fatima et al., 2013; Klowden, 2013). Being involved in phagocytosis and encapsulation, haemocyte forms the cellular immune system of many arthropods (Gupta, 2019; Salt, 1970). Haemolymph of insects contains number of circulating cells ranging from 25000-100,000 per cubic mm but this range of cells is much more less than the large insects (Fatima et al., 2016).

When a chemical is applied on insect, first of all it affects the defense system of insect pests by altering its haemocytes number. Different insects have shown different effects on circulating cell types and shapes when different insecticides were applied on them (Qamar and Jamal, 2009). Insects use the immunity system to counteract infections by a diverse array of pathogens including the identification of pathogens connected with molecular pattern (Janeway, 1989). Even when insects encounter a pesticide particle, they trigger their immune system and hence the pesticide, which is capable to suppress the immune system efficiently, is considered good. Therefore, the present study was conducted to evaluate the efficiency of new chemistry insecticides on the total and differential haemocytes counts of maize stem borer and to evaluate the abnormalities in the haemocytes of maize stem borer.

## MATERIALS AND METHODS

**Insect culture:** In the present study, the already established culture of maize stem borer (*Chilo partellus*) was used. The rearing method of stem borer included 10 larva per jar which was kept with newly cut the maize stem for food. The piece of maize stem was put into the

jar and then was tight head of jar with muslin cloth. The food of larvae was changed after 2 days' interval as per need, larvae were observed for pupation while changing food pupae were collected and kept in emergence jar. Pupae were observed on daily basis for emergence.

Adults on emergence were shifted to oviposition jars. Ovipositor jars were lined with white paper at bottom and with leaves of maize plant at sides. Leaves were placed for egg laying by adult moths. Leaves were changed on daily basis. Eggs were collected by cutting leaves and white paper into small pieces and were shifted in hatching jar. After 4-6 days first instars, larvae hatched from eggs and were shifted to rearing jars with food. The cycle goes on for mass culturing. Insect culture was maintained at  $25 \pm 2^\circ\text{C}$  with  $60 \pm 5\%$  relative humidity.

**Insecticides:** In the present study, four new chemistry insecticides were used to evaluate the haemocytological activity of *Chilo partellus*.  $LC_{50}$  and  $LC_{60}$  of each insecticide were calculated using bioassay. Following new chemistry insecticide were used for the experiment. Radiant 120 SC (Spinetoram)

Chlorfenapyr 360 SC (Chlorfenapyr)

Belt 48 S C (Flubendiamide)

Match 50 EC (Lufenuron)

**Bioassays:** To calculate the lethal concentration values, natural diet of maize stem was used by diet incorporation bioassay method. Five concentrations of each insecticide were mixed in 10 ml of water in five separate beakers. Then freshly prepared diet was poured in each beaker making a total weight of 100 ml (10 ml water: 90ml diet). In the control beaker, no insecticides were mixed but just the distilled water. In the diet preparation, antibiotic mixture was not used when the diet was prepared for bioassay and a reduction of 10 percent insecticides mixture can easily solidify the natural diet mixture.

Each concentration was fed to 30 insects larvae in the six whole petri-plates and data were taken after 24 hr 48 hr And 72 hr. Mortality for each concentration were observed and a value of lethal concentration were calculated by using R statistical program. Similar, procedure was used for all other insecticides on 3<sup>rd</sup> and 5<sup>th</sup> instars larvae.  $LC_{50}$  and  $LC_{60}$  were calculated out using the R version 2.9.0 of all the four insecticides against selected instar larvae.

**Insecticides application:** The insecticide was applied on individual insect with the help of micro applicator at

the rate of 3 ul per larvae. Third and fifth larval instars were chosen for insecticide application and haemocytes studies. Effect of these insecticides was observed immediately after application, after 30 minutes and after 60 minutes in terms of abnormalities in total haemocyte count and differential haemocyte count.

**Preparation of slides:** The haemolymph of *Chilo Partellus* larvae were taken on a microscopic slide with help of insulin syringe from the abdominal of larvae. A smear was made by drawing a second slide across the first one. This smear was air dried for four minutes and stained properly with Wright's stain by following procedure.

- a) Air dried the blood film for four minutes
- b) Dip the slide having blood smear in methyl alcohol for 5-7 minutes
- c) Air dried the film
- d) Immerse the dried film in the Wright's stain for 15 minutes
- e) Wash the slides with the distilled water of pH 6.8
- f) Air dried the slides
- g) Neutralize the haemocyte content in freshly prepared buffer solution of pH 6.6 for 15 minutes

The slides were mounted with the Canada balsam and put a glass cover slip on the slide to observe blood types under microscope.

**Differential haemocytes count (DHC):** After making the slides, different types of haemocytes were identified within the hemolymph of *Chilo partellus*. Differential haemocytes counting were done with the help of telecounter under Phase Contrast microscope 10X. A minimum of 200 cells were counted each time and the percentage of various classes were calculated.

**Total haemocytes count (THC):** Haemocytometer were used for the counting of total haemocytes. Standard sampling of the haemolymph was carried out with white blood cell diluting pipette. Haemolymph for the abdominal legs were collected on a glass slide and then quickly drawn into white blood cell diluting pipette upto mark 0.5. And then diluted 20 times with toissin (NaCl = 1.0 gm, Na<sub>2</sub>SO<sub>4</sub> = 8.0 gm, neutral glycerine = 30ml, Methyl violet =0.025 gm and distilled water =160ml) and Thoma white blood cell diluting pipette was filled upto mark This solution was properly stained with staining shaker for 5 minutes it prevents the blood to coagulate.

Three initial drop of haemolymph mixture were placed near the edge of the coverslip of the Neubauer

haemocytometer. The counting chamber was filled automatically by capillary action. Haemocytometer will leave for 5 minutes so that the blood cell could settle down and then observed the haemocytes under phase contract microscope 10X. The four corners square of both chamber of haemocytometer were counted under low power followed by high power of microscope focusing the counting chamber.

Cells of each group of 16 squares, touching bordering the bottom left hand side and the central line were counted. Cells touching the central line in the top and right hand side will not include in the count. Total haemocytes counting (THC) were done following the formula described by Jones (1962). Actual number of cell per cubic mm = Average number of cell counted per square millimeter x depth x dilution per square millimeter.

**Statistical analysis:** The treatment (insecticide doses) were replicated four times using CRD (Completely

Randomized Design) and for each treatment, 30 insects were used with five replications. Six individuals were selected randomly and taking average as one reading for each of the replicate.

## RESULTS AND DISCUSSION

**Toxicity bioassays:** The current study was carried out to evaluate the toxicity of four new chemistry insecticides and its effect on haemocytological activities of the 3<sup>rd</sup> and 5<sup>th</sup> instar larvae of *C. partellus*. The LC<sub>50</sub> and LC<sub>60</sub> values for Radiant against 3<sup>rd</sup> instar of *C. partellus* after 24 hours were 12.2 ppm and 24.2 ppm respectively (Table 1), while the same value after 72 hours was 7.24 ppm and 14.84 ppm respectively. For the 5<sup>th</sup> larval instar of *C. partellus*, the LC<sub>50</sub> and LC<sub>60</sub> values were calculated as 44.2 ppm and 61.9 ppm respectively (Table 2) after 24 hours and LC<sub>50</sub> and LC<sub>60</sub> values for radiant against 5<sup>th</sup> larval instar of *C. partellus* were calculated as 23.94 ppm and 43.51 ppm respectively after 72 hours.

Table 1: LC<sub>50</sub> and LC<sub>60</sub> values of different new chemistry insecticides for 3<sup>rd</sup> instar larvae of *Chilo partellus* after 24, 48 and 72 h.

Insecticides	N	Time (h)	LC <sub>50</sub> (ppm) (95% CL)	LC <sub>60</sub> (ppm) (95% CL)	Slope(±SE)
Radiant	30	24	12.2 (3.19-22.81)	24.2 (12.1-36.27)	1.47 (±0.106)
	30	48	10.14 (2.11-16.1)	18.24 (9.12-30.17)	1.97 (±0.100)
	30	72	7.24 (2.2-13.2)	14.84 (4.53-24.26)	1.46 (±0.112)
Chlorfenapyr	30	24	8.21 (3.52-16.27)	16.35 (7.48-27.29)	1.90 (±0.093)
	30	48	6.19 (1.48-14.24)	12.57 (5.37-21.4)	1.67 (±0.108)
	30	72	4.21 (1.08-10.17)	7.31 (3.17-12.22)	1.86 (±0.118)
Belt	30	24	3.4 (0.99-5.89)	7.14 (3.9-13.2)	1.77 (±0.106)
	30	48	1.99 (0.049-4.28)	3.23 (1.002-7.27)	1.79 (±0.101)
	30	72	0.152 (0.011-2.23)	1.78 (0.079-4.87)	1.94 (±0.112)
Match	30	24	11.21 (5.5-21.27)	21.2 (12.1-30.9)	1.99 (±0.093)
	30	48	7.94 (2.54-18.25)	14.73 (6.64-24.53)	1.37 (±0.108)
	30	72	3.126 (0.948-10.1)	8.65 (3.27-19.22)	1.93 (±0.118)

Chlorfenapyr application against the 3<sup>rd</sup> larval instar showed higher LC<sub>50</sub> and LC<sub>60</sub> values as 8.21 ppm and 16.35 ppm (Table 1) after 24 hours, while after 72 hours these values decreased as 4.21 ppm and 7.31 ppm for LC<sub>50</sub> and LC<sub>60</sub> respectively. Similarly, the LC<sub>50</sub> and LC<sub>60</sub> values for 5<sup>th</sup> larval instar of *C. partellus* were 56.4 ppm and 73.51 ppm respectively (Table 2). After 72 hours, LC<sub>50</sub> and LC<sub>60</sub> were calculated as 32.91 ppm and 52.71 ppm respectively.

Results revealed that among the four new chemistry

insecticides used in the current study, Belt was much more effective as compared to others. The LC<sub>50</sub> and LC<sub>60</sub> values for 3<sup>rd</sup> larval instar were calculated as 3.4 ppm and 7.14 ppm after 24 hours (Table 1), with respect to time decrease was observed in the LC<sub>50</sub> and LC<sub>60</sub> values after 72 hours as 0.152 ppm and 1.78 ppm respectively. For 5<sup>th</sup> larval instar the LC<sub>50</sub> and LC<sub>60</sub> values were calculated as 21.2 ppm and 36.7 ppm after 24 hours (Table 2) and after 72 hours these values were observed as 8.25 ppm and 14.5 ppm respectively.

Table 2: LC<sub>50</sub> and LC<sub>60</sub> values of different new chemistry insecticides for 5<sup>th</sup> instar larvae of *Chilo partellus* after 24, 48 and 72 h.

Insecticides	N	Time (h)	LC <sub>50</sub> (ppm)	LC <sub>60</sub> (ppm)	Slope(±SE)
Radiant	30	24	44.2	61.9	1.91 (±0.127)
	30	48	32.7	52.64	1.78 (±0.140)
	30	72	23.94	43.51	1.62 (±0.172)
Chlorfenapyr	30	24	56.4	73.51	1.94(±0.093)
	30	48	42.29	63.17	1.87 (±0.128)
	30	72	32.91	52.71	1.97 (±0.121)
Belt	30	24	21.2	36.7	1.81 (±0.096)
	30	48	16.29	25.93	1.90 (±0.124)
	30	72	8.25	14.5	1.84 (±0.108)
Match	30	24	39.5	59.7	1.88 (±0.093)
	30	48	24.14	41.53	1.67 (±0.121)
	30	72	18.26	33.45	1.73 (±0.127)

Match was also used against the 3<sup>rd</sup> and 5<sup>th</sup> larval instar of *C. partellus* in the present study. Results of the 3<sup>rd</sup> larval instar were observed as LC<sub>50</sub> of 11.21 ppm and LC<sub>60</sub> of 21.2 ppm after 24 hours (Table 1), while after 72 hours the results were as LC<sub>50</sub> of 3.126 ppm and LC<sub>60</sub> of 8.65 ppm respectively. The results for 5<sup>th</sup> larval instar, LC<sub>50</sub> of 39.5 ppm and LC<sub>60</sub> of 59.7 ppm after 24 hours and LC<sub>50</sub> of 18.26 ppm and LC<sub>60</sub> of 33.45 ppm after 72 hours (Table 2) were observed respectively.

The above results showed that Belt was more effective against the 3<sup>rd</sup> and 5<sup>th</sup> larval instars of *C. partellus* on the basis of their low LC<sub>50</sub> and LC<sub>60</sub> values as compared to the other tested three insecticides. The reason for this low LC values might be that Belt is toxic to borers while other insecticides are effective against the sucking insects.

**Observed types of haemocytes:** On the basis of their essential characteristics, following six different haemocytes were observed in the haemolymph of *C. partellus*.

Prohaemocytes (PR)

Plasmatocytes (PL)

Spherulocytes (SP)

Granulocytes (GR)

Cystocytes (CO)

Oenocytoids (OE)

**Total and differential haemocytes count for 3<sup>rd</sup> larval instar of *C. partellus*:** after the application of all four insecticides on the 3<sup>rd</sup> instar larvae of *c. partellus*, the total haemocytes and differential haemocytes were observed and calculated as prescribed earlier.

**Total and differential haemocytes counts in untreated:** in the current study, the mean counts of total haemocytes observed were about 72155 blood cells/mm<sup>3</sup> in the 3<sup>rd</sup> larval instar of *c. partellus*. The percent of different haemocytes in untreated 3<sup>rd</sup> instar larvae of *c. partellus* were observed as prohaemocytes (43.23%), spherulocytes (31.388%), plasmatocytoid (17.86%), granulocytes (9.12%) and cystocytes (4.22%).

**Total haemocytes and differential haemocytes for Radiant at LC<sub>50</sub>:** Table 3 shows the total haemocytes count of the 3<sup>rd</sup> larval instar of *C. partellus* when treated with Radiant. The finding showed that the total haemocytes counts increased (75841 cells/mm<sup>3</sup>) from the normal (72155 cells/mm<sup>3</sup>) just after the application of insecticides radiant, after 30 minutes the total haemocytes count decreases (71049.5 cells/mm<sup>3</sup>), while after 60 minutes, the total Haemocytes count again increases (79248 cell/mm<sup>3</sup>). The current findings are in agreement with Fatima et al. (2013) and Fareed (2001) who noted that the total haemocytes increased immediately after the application of the chemical, then decreases and again after one hour the haemocytes increases.

Table 4 illustrates the differential haemocytes count observed in the 3<sup>rd</sup> larval instar of *C. partellus*. Differential haemocytes counts, the percent of plasmocytes have increase from 17.86% to 28 %, however the percent of spherulocytes, granulocytes, prohaemocytes and cystocytes have decreases from the untreated value as 30%, 8%, 31% and 3% respectively. These results are in favor to the results of Alhariri et al. (2002), Fatima et al. (2013) and Fareed (2001) who stated that the plasmocytes increases with the application of insecticides, however our results are dissimilar to Rizwan-ul-Haq et al. (2005) who found that after the application of imidacloprid the percent of plasmocytod decreases from its normal untreated percentage.

Table 3: Total haemocytes counts of *C. partellus* larvae affected by Radiant.

Radiant Conc.	LC <sub>50</sub>		LC <sub>60</sub>	
	3 <sup>rd</sup> instar	5 <sup>th</sup> instar	3 <sup>rd</sup> instar	5 <sup>th</sup> instar
0 Minutes	75841	22541.5	78548.5	26485
30 Minutes	71049.5	18543	71694	19620
60 Minutes	79248	24567	80215	27940

Table 4: Differential haemocytes counts of *C. partellus* larvae affected by Radiant.

Radiant Conc.	LC <sub>50</sub>		LC <sub>60</sub>	
	3 <sup>rd</sup> instar	5 <sup>th</sup> instar	3 <sup>rd</sup> instar	5 <sup>th</sup> instar
PR%	31	29	33	30.5
PL%	28	31.25	26	27.75
SP%	30	26	39	28.5
GR%	8	4	6.75	2
CO%	3	3.75	2.25	1.25
OE%	0	6	0	4

**Total haemocytes and differential haemocytes for Radiant at LC<sub>60</sub>:** After the application of Radiant insecticide, the total haemocytes count immediately increase (78548.5 cells/mm<sup>3</sup>) as compared to normal (71155 cells/mm<sup>3</sup>), however after 30 minutes to decreases to (71694 cells/mm<sup>3</sup>) and after 60 minutes of interval, the total haemocytes count again increases as (80215 cell/mm<sup>3</sup>) (Table 3). Percent plasmocytes increase from untreated to 26%, while on 3<sup>rd</sup> larval instar of *C. partellus* the spherulocytes, granulocytes, prohaemocytes and cystocytes also fluctuated from the untreated as 39%, 6.75%, 33% and 2.25% respectively (Table 4).

**Total haemocytes and differential haemocytes for Chlorfenapyr at LC<sub>50</sub>:** Table 5 shows the total

haemocytes count of the 3<sup>rd</sup> larval instar of *C. partellus* when treated with chlorfenpyr. The finding show that the total haemocytes counts increased (82640 cells/mm<sup>3</sup>) from the normal (72155 cells/mm<sup>3</sup>) just after the application of insecticides Chlorfenapyr, after 30 minutes the total haemocytes count decreases (72010 cells/mm<sup>3</sup>), while after 60 minutes, the total haemocytes count again increases (86350 cell/mm<sup>3</sup>). The current findings are in agreement with Abbas et al. (2008) who used Sevin and Match respectively on *Papilio demoleus* and found that the total haemocytes increased immediately after the application of the chemical, then decreased and again after one hour the haemocytes increased.

Table 6 illustrates the differential haemocytes count observed in the 3<sup>rd</sup> larval instar of *C. partellus*. Differential haemocytes counts, the percent of plasmocytes have increased from 17.86% to 30.25%, however the percent of spherulocytes, granulocytes, prohaemocytes and cystocytes have decreased from the untreated value as 26.5%, 6.5%, 32.75% and 1% respectively. These results are in favor to the results of Abbas et al. (2008) and Fareed (2001) who stated that the plasmocytes increases with the application of insecticides.

#### Total haemocytes and differential haemocytes for

**Chlorfenapyr at LC<sub>60</sub>:** After the application of Chlorfenapyr insecticide, the total haemocytes count immediately increased (80920.75 cells/mm<sup>3</sup>) as compared to normal (71255 cells/mm<sup>3</sup>), however after 30 minutes decreased to (71155 cells/mm<sup>3</sup>) and after 60 minutes of interval, the total haemocytes count again increased as (84100 cell/mm<sup>3</sup>) (Table 5). Percent plasmotocytes increased from untreated to 36%, while the spherulocytes, granulocytes, prohaemocytes and cystocytes also fluctuated from the untreated as 23.5%, 5.5%, 29% and 1.5% respectively (Table 6).

Table 5: Total Haemocytes counts of *C. partellus* larvae affected by Chlorfenapyr.

Conc.	LC <sub>50</sub>		LC <sub>60</sub>	
	3 <sup>rd</sup> instar	5 <sup>th</sup> instar	3 <sup>rd</sup> instar	5 <sup>th</sup> instar
0 Minutes	82640	22435	80920.75	23120
30 Minutes	72010	18300	71155	20100
60 Minutes	86350	24595	84100	25600

Table 6: Differential Haemocytes counts of *C. partellus* larvae affected by Chlorfenapyr.

Conc.	LC <sub>50</sub>		LC <sub>60</sub>	
	3 <sup>rd</sup> instar	5 <sup>th</sup> instar	3 <sup>rd</sup> instar	5 <sup>th</sup> instar
PR%	32.75	30	29	27
PL%	30.25	31.5	36	44
SP%	26.5	19	23.5	17
GR%	6.5	1.75	5.5	1.25
CO%	1	0.5	1.5	0.75
OE%	0	12.25	0	11

**Total haemocytes and differential haemocytes for Belt at LC<sub>50</sub>:** Table 7 shows the total haemocytes count of the 3<sup>rd</sup> larval instar of *C. partellus* when treated with Belt. The finding showed that the total haemocytes counts increased (82500 cells/mm<sup>3</sup>) from the normal (72155 cells/mm<sup>3</sup>) just after the application of insecticides Belt, after 30 minutes the total haemocytes count was (70200 cells/mm<sup>3</sup>), while after 60 minutes, the total haemocytes count again increased (84550 cell/mm<sup>3</sup>). The current findings are in agreement with those of Fatima et al. (2013); Rajak et al. (2014) and Fareed (2001) who noted that the total haemocytes increased immediately after the application of the chemical, then decreased and again after one hour the haemocytes increases.

Table 8 shows the differential haemocytes count observed in the 3<sup>rd</sup> larval instar of *C. partellus*.

Differential haemocytes counts, the percent of plasmocytes have increased from 17.86% to 31%, however the percent of spherulocytes, granulocytes, prohaemocytes and cystocytes have decreased from the untreated value as 29%, 9%, 28% and 4% respectively. These results are in agreement with the results of Alhariri et al. (2002) and Fatima et al. (2014); Fatima et al. (2016) who stated that the plasmocytes increased with the application of insecticides, however our results are dissimilar to Kalyani and Holihosur (2015) who found that after the application of chemical the percent of plasmocytes decreased from its normal untreated percentage.

**Total haemocytes and differential haemocytes for Belt at LC<sub>60</sub>:** After the application of Belt insecticide, the total haemocytes count immediately increased (87200 cells/mm<sup>3</sup>) as compared to normal (71255 cells/mm<sup>3</sup>),

however after 30 minutes decreased to (71270 cells/mm<sup>3</sup>) and after 60 minutes of interval, the total haemocytes count again increased as (89640 cell/mm<sup>3</sup>) (Table 7). Percent plasmatocytes increased from

untreated to 36%, while the spherulocytes, granulocytes, prohaemocytes and cystocytes also fluctuated from the untreated as 32.5%, 8.5%, 17.25% and 4.75% respectively (Table 8).

Table 7: Total haemocytes counts of *C. partellus* larvae affected by Belt.

Conc.	LC <sub>50</sub>		LC <sub>60</sub>	
	3 <sup>rd</sup> instar	5 <sup>th</sup> instar	3 <sup>rd</sup> instar	5 <sup>th</sup> instar
0 Minutes	82500	19500	87200	21535
30 Minutes	70200	18965	71270	18532.5
60 Minutes	84550	21055	89640	23540

Table 8: Differential Haemocytes counts of *C. partellus* larvae affected by Belt.

Conc.	LC <sub>50</sub>		LC <sub>60</sub>	
	3 <sup>rd</sup> instar	5 <sup>th</sup> instar	3 <sup>rd</sup> instar	5 <sup>th</sup> instar
PR%	28	35.5	17.25	23
PL%	31	32.25	36	38
SP%	29	25	32.5	28.5
GR%	9	5.5	8.5	6.5
CO%	4	3	4.75	4
OE%	0	13.75	0	12

**Total haemocytes and differential haemocytes for Match at LC<sub>50</sub>:** Table 9 shows the total haemocytes count of the 3<sup>rd</sup> larval instar of *C. partellus* when treated with match. The finding showed that the total haemocytes counts increased (78375 cells/mm<sup>3</sup>) from the normal (72155 cells/mm<sup>3</sup>) just after the application of insecticides Match, after 30 minutes the total haemocytes count decreased (72080 cells/mm<sup>3</sup>), while after 60 minutes, the total haemocytes count again increased (86500 cell/mm<sup>3</sup>). The current findings are in agreement with Abbas et al. (2008) who used Match respectively on *Papilio demoleus* and found that the total haemocytes

increased immediately after the application of the chemical and then decreased and again after one hour the haemocytes increased. Table 10 illustrates the differential haemocytes count observed in the 3<sup>rd</sup> larval instar of *C. partellus*. Differential Haemocytes counts, the percent of plasmatocytes have increased from 17.86% to 28 %, however the percent of spherulocytes, granulocytes, prohaemocytes and cystocytes have decreased from the untreated value as 33%, 11%, 27% and 5% respectively. These results are in line with those of Abbas et al. (2008) and Fareed (2001) who stated that the plasmatocytes increased with the application of insecticides.

Table 9: Total Haemocytes counts of *C. partellus* larvae affected by Match.

Conc.	LC <sub>50</sub>		LC <sub>60</sub>	
	3 <sup>rd</sup> instar	5 <sup>th</sup> instar	3 <sup>rd</sup> instar	5 <sup>th</sup> instar
0 Minutes	78375	15600	87500	16565
30 Minutes	72080	12245	72190	15600
60 Minutes	86500	19865	99800	23855

**Total haemocytes and differential haemocytes for Match at LC<sub>60</sub>:** After the application of match insecticide, the total haemocytes count immediately increased (87500 cells/mm<sup>3</sup>) as compared to normal (72155

cells/mm<sup>3</sup>), however after 30 minutes decreased to (72190 cells/mm<sup>3</sup>) and after 60 minutes of interval, the total haemocytes count again increased as (99800 cell/mm<sup>3</sup>) (Table 9). Percent plasmatocytes increased



from untreated to 33%, while the spherulocytes, granulocytes, prohaemocytes and cystocytes also fluctuated from the untreated as 31%, 14%, 23% and 4 % respectively (Table 10).

Table 10 Differential Haemocytes counts of *C. partellus* larvae affected by Match.

Conc.	LC <sub>50</sub>		LC <sub>60</sub>	
	3 <sup>rd</sup> instar	5 <sup>th</sup> instar	3 <sup>rd</sup> instar	5 <sup>th</sup> instar
PR%	27	31	23	29
PL%	28	29	33	32
SP%	33	34	31	30
GR%	11	9.25	14	11.75
CO%	5	6.75	4	5.25
OE%	0	14	0	10

#### Author's contribution

SA, MT and AG designed the study; SA, RA and SK executed the experimental work; AG and MI analyzed the data; MT and MI supervised the work; SA wrote the manuscript; MT, AG and SK edited the manuscript and all the authors approved the write-up.

#### Conflict of interest

The authors declare no conflict of interest.

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