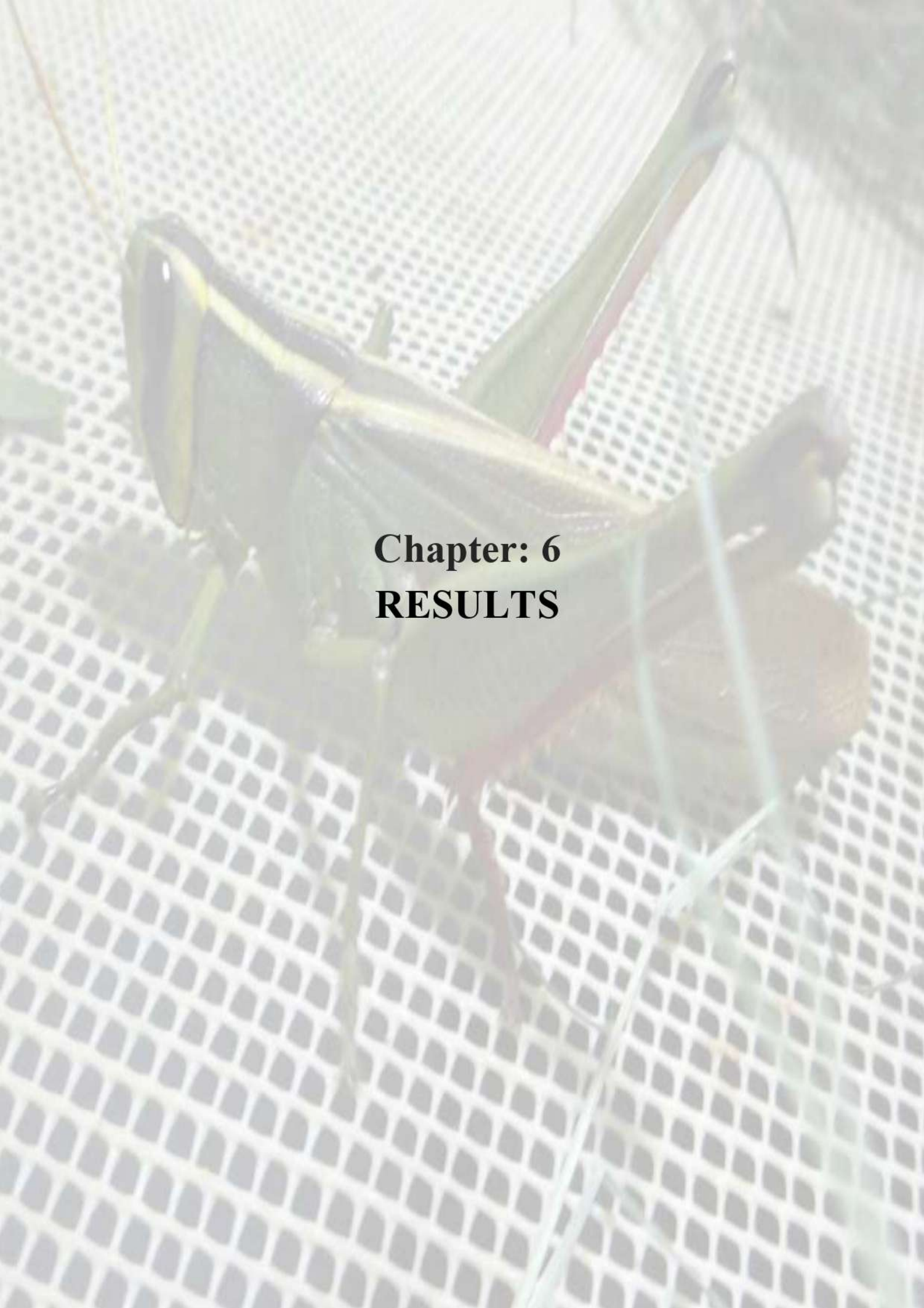

Chapter: 6
RESULTS

6. RESULTS

The screening toxicity study was conducted to assess the pesticidal efficacy of commonly used pesticides in-vitro tissue of common agri-field animals. The toxicological efficacy studies of azadirachtin (AZT) on different organs of *Spathosternum prasiniferum prasiniferum* were carried out in the present investigation.

6.1. Screening toxicity studies on common agri-field animals by widely used pesticide

6.1.1. Oxidative stress (OS) induced in-vitro toxicity in common agri-field animals

In general, all pesticides were found to impair enzymatic activities. In this study the catalase (CAT) activity in goat liver decreased after in-vitro incubation with pesticides and this inhibition was prominent in case of chlorpyrifos (Fig. 1.a) and AZT (Fig. 1.c). The activity of superoxide-dismutase (SOD) in goat liver distinctly decreased after chlorpyrifos intoxication (Fig. 1.f). However, in case of gut tissues in insect, both CAT and SOD activities increased after in-vivo and in-vitro AZT exposure which is found to be highly significant (Fig. 1.d and e and Fig. 1.i and j). Moreover, the extent of increased enzymatic activities with comparison to control is higher in female insect (lane 1-6) than that of male insect (lane 7-12). An appreciable amount of decrease in SOD activity was noticed in the liver of cattle (Fig. 1.f and 1.g). Nevertheless, a significant dose dependant increase of SOD activity was noticed after both in-vivo and in-vitro AZT exposure to the insect of both sexes.

6.1.2. Oxidative stress induced DNA stability/toxicity in common agri-field animals

In present study, a significant DNA-laddering was found in the experimental insect induced by AZT (Fig. 2.j and 2.k). A moderate DNA-laddering was also noticed in the bird-hen (Fig. 2.g, 2.h and 2.i) for three pesticides. Nevertheless, in the higher vertebrate, DNA was found to be more stable (Fig. 2.a to 2.f).

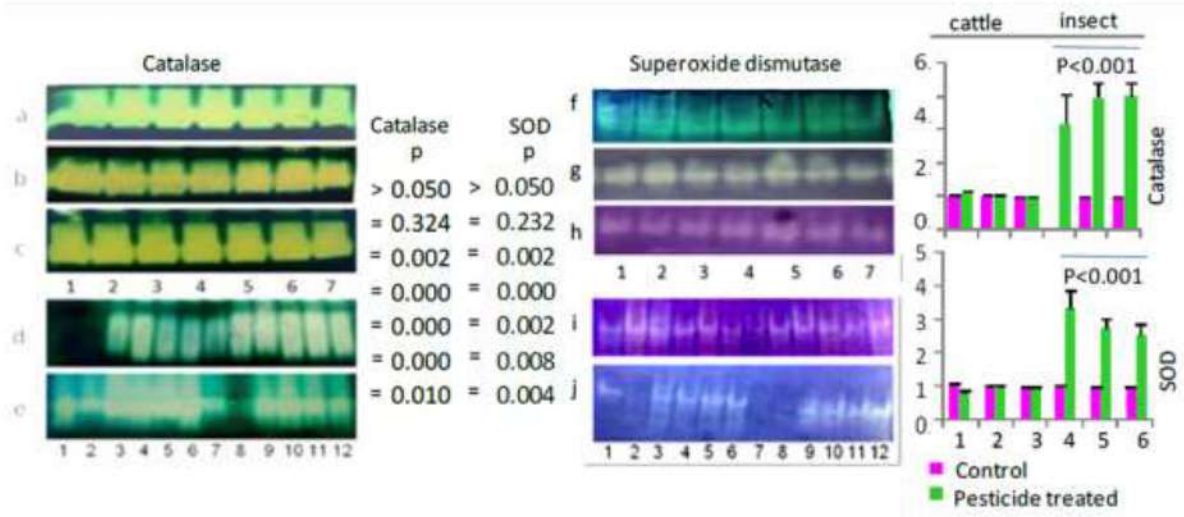

Fig. 1. Catalase and SOD activity in-gel-zymogram of mammalian in-vitro hepatic tissue (treated with chlorpyrifos/fenvalerate/nimbecidine) and insect in-vivo and in-vitro gut (treated with nimbecidine) **Left panel:** Goat (*Capra aegagrus hircus*) liver CAT activity in-gel-zymogram. **gel a-** CAT activity in goat liver treated with chlorpyrifos (Lane: 1,2- control, 3- 0.20%, 4- 0.27%, 5- 0.40%, 6- 0.80%, 7- 4.00%); **gel b-** CAT activity in goat liver treated with fenvalerate (Lane: 1,2- control, 3- 0.08%, 4- 0.10%, 5- 0.13%, 6- 0.20%. 7- 0.40%); **gel c-** CAT activity in goat liver treated with nimbecidine (Lane: 1,2- control, 3- 100 ppm, 4- 133 ppm, 5- 200 ppm, 6- 400 ppm, 7- 2000 ppm); **gel d-** CAT activity is shown in the gut of insect in-vivo treated with nimbecidine, Lane 1-6 female gut (1- control, 2- 1 ppm, 3- 5 ppm, 4- 10 ppm, 5- 15 ppm, 6- 20 ppm) and Lane 7-12 male gut (7- control, 8- 1 ppm, 9- 5 ppm, 10- 10 ppm, 11- 15 ppm, 12- 20 ppm); **gel e-** CAT activity is shown in the gut of insect in-vitro treated with nimbecidine, Lane 1-6 female gut (1- control, 2- 1 ppm, 3- 5 ppm, 4- 10 ppm, 5- 15 ppm, 6- 20 ppm) and Lane 7-12 male gut (7- control, 8- 1 ppm, 9- 5 ppm, 10- 10 ppm, 11- 15 ppm, 12- 20 ppm). **Right panel:** Superoxide-dismutase activity is shown on a polyacrylamide gel- Lane distribution in gel **f, g, h, i** and **j** are similar to gel **a, b, c, d** and **e** in the right panel respectively. Data in the last panel is the mean ± SE. Statistical data-P values of comparison between each drug-dose group of cattle and insects. Right most figure of bar-diagram comparison of all drugs together in cattle versus insect. Statistical data shows that insects are more influenced and affected by pesticide exposure.

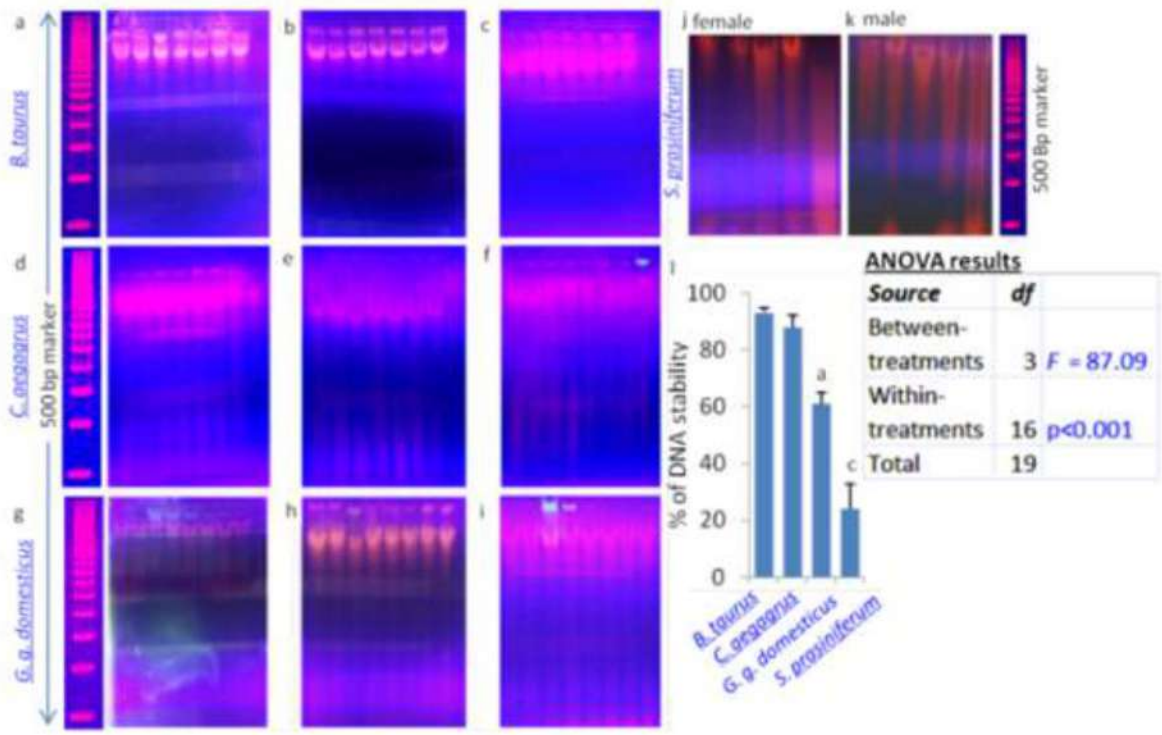

Fig. 2. DNA stability (%) of mammalian and bird in-vitro hepatic tissue (treated with chlorpyrifos/fenvalerate/nimbecidine) and insect in-vivo and in-vitro gut (treated with nimbecidine). **(a, b, c)** DNA-fragmentation result is shown in the liver of cow (*Bos taurus*) in-vitro treated with chlorpyrifos **(a)** Lane: 1,2- control, 3- 0.20%, 4- 0.27%, 5- 0.40%, 6- 0.80%. 7- 4.00%; fenvalerate **(b)** Lane: 1,2- control, 3- 0.08%, 4- 0.10%, 5- 0.13%, 6- 0.20%. 7- 0.40%; nimbecidine (AZT) **(c)** Lane: 1,2- control, 3- 100 ppm, 4- 133 ppm, 5- 200 ppm, 6- 400 ppm. 7- 2000 ppm. **(d, e, f)** DNA-fragmentation result is shown in the liver of goat (*Capra aegagrus hircus*) in-vitro treated with chlorpyrifos **(d)** Lane: 1,2- control, 3- 0.20%, 4- 0.27%, 5- 0.40%, 6- 0.80%, 7- 4.00%; flavaralate **(e)** Lane: 1,2- control, 3- 0.08%, 4- 0.10%, 5- 0.13%, 6- 0.20%. 7- 0.40%; nimbecidine (AZT) **(f)** Lane: 1,2- control, 3- 100 ppm, 4- 133 ppm, 5- 200 ppm, 6- 400 ppm. 7- 2000 ppm. **(g, h, i)** DNA-fragmentation result is shown in the liver of bird-hen (*Gallus gallus domesticus*) in-vitro treated with chlorpyrifos **(g)** Lane: 1,2- control, 3- 0.20%, 4- 0.27%, 5- 0.40%, 6- 0.80%, 7- 4.00%; fenvalerate **(h)** Lane: 1,2- control, 3- 0.08%, 4- 0.10%, 5- 0.13%, 6- 0.20%. 7- 0.40%; nimbecidine **(i)** Lane: 1,2- control, 3- 100 ppm, 4- 133 ppm, 5- 200 ppm, 6- 400 ppm, 7- 2000 ppm. DNA-fragmentation result is shown in-vivo gut treated with nimbecidine (AZT) **(j)** female gut, Lane: 1- control, 2- 1 ppm, 3- 5 ppm, 4- 10 ppm, 5- 15 ppm **(k)** male gut, Lane: 1- control, 2- 1 ppm, 3- 5 ppm, 4- 10 ppm, 5- 15 ppm. Bar-diagram results (5 independent experiments) and Student's t-test values suggest that insects are the most affected and then the birds' group in terms of their DNA stability. Level of significances are shown; **a.** $P<0.05$ and **c.** $P<0.001$. ANOVA result suggests that between and within groups, the differences in the DNA stability are significantly different at $P<0.001$ ($F=87.09$).

6.1.3. Oxidative stress induced in-vitro sub-cellular toxicity in common agri-field animals

The mitochondrial membrane degeneration reflected in present oxidative stress (OS) induced cellular toxicity studies (Fig. 3). Mitochondrial stability and function has not been observed in those previous experiments. In the current investigation, we evaluated mitochondrial membrane potential in cattle and bird-hen and considered that pesticides have a strong destabilizing activity.

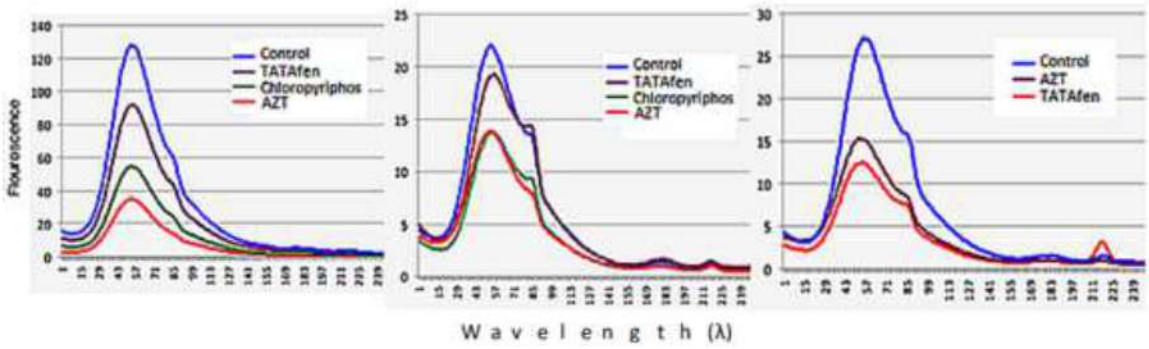

Fig. 3. Mitochondrial membrane potential/stability assays (Rhodamine method) by fluorescence microscopy. Depending on the sample availability, this experiment was conducted (Left panel: cow, middle panel: goat and right panel: bird-hen).

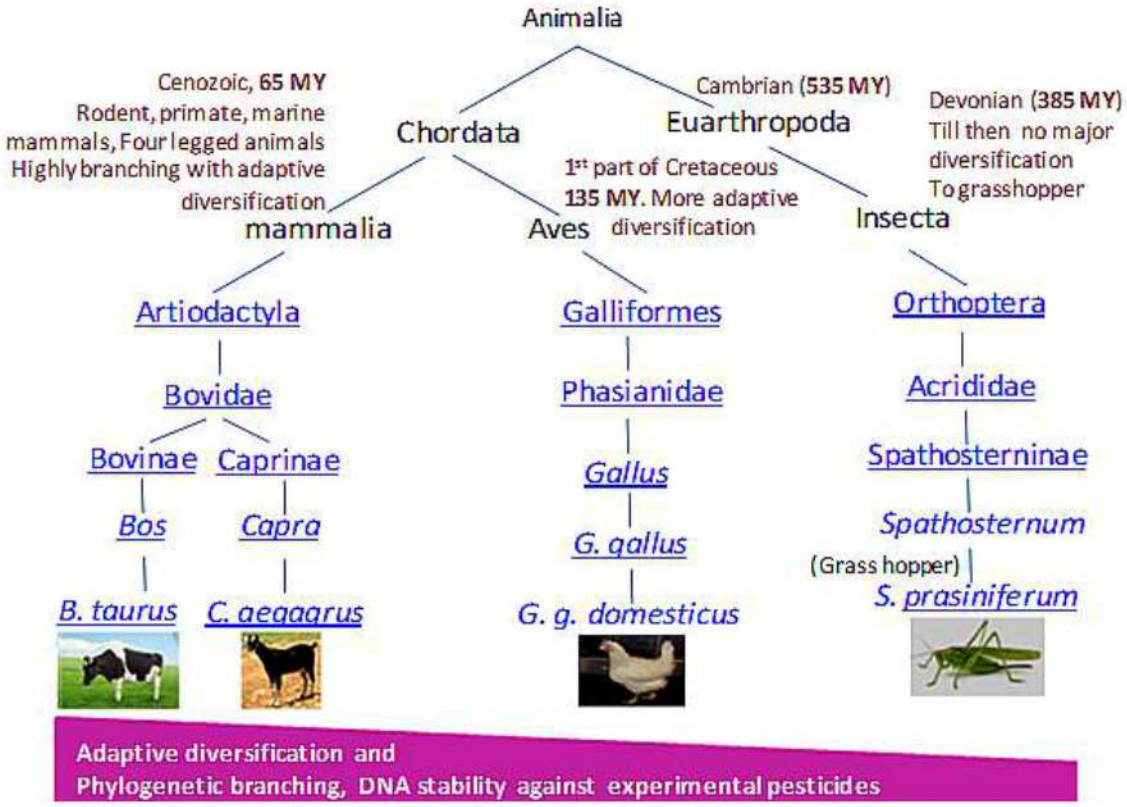

Plate 23. Respective position and lineage diversification of four common living agri-field animals investigated in the screening toxicity study; cow, goat, bird-hen and an insect-grasshopper. DNA stability has been shown to be related to the relative phylogenetic position of the organism.

Table 3. Biodiversity of acridid species (short-horned-grasshopper) in Midnapore (East and West) district, West Bengal.

Sl. No	Species
1	*Acorypha glaucopsis* (Walker, 1870)
2	*Acrida exaltata* (Walker, 1859)
3	*Acrida gigantea* (Herbst, 1786)
4	*Acrida turrita* (Linnaeus, 1758)
5	*Acrotylus humbertianus* (Saussure, 1884)
6	*Acrotylus insubricus insubricus* (Scopoli, 1786)
7	*Aiolopus simulatrix* (Walker, 1870)
8	*Aiolopus thalassinus tamulus* (Fabricius, 1798)
9	*Atractomorpha crenulata* (Fabricius, 1793)
10	*Aulacobothrus luteipes infernus* (Bolivar, 1902)
11	*Catantops erubescens* (Walker, 1870)
12	*Ceracris nigricornis nigricornis* (Walker, 1870)
13	*Chondacris rosea* (De Geer, 1773)
14	*Chrotogonus trachypterus* (Blanchard, 1836)
15	*Cyrtacanthacris tatarica tatarica* (Linnaeus, 1758)
16	*Eucoptacra binghami* (Uvarov, 1921)
17	*Eucoptacra praemorsa* (Stal, 1861)
18	*Eyprepocnemis alacris alacris* (Serville, 1838)
19	*Gasonula punctiforns* (Stal, 1861)
20	*Gastrimargus africanus africanus* (Saussure, 1888)
21	*Heteracris littoralis* (Rambur, 1838)
22	*Hieroglyphus nigrorepletus* (Bolivar, 1912)
23	*Hieroglyphus oryzivorus* (Carl, 1961)
24	*Locusta migratoria migratoria* (Linnaeus, 1758)
25	*Morphacris fasciata* (Thunberg, 1815)
26	*Oedaleus abruptus* (Thunberg, 1815)
27	*Orthochtha indica* (Uvarov, 1942)
28	*Oxya fuscovittata* (Marschall, 1836)
29	*Oxya hyla hyla* (Serville, 1831)
30	*Oxya japonica japonica* (Thunberg, 1815)
31	*Oxya velox* (Fabricius, 1787)
32	*Phlaeoba angustidorsis angustidorsis* (Bolivar, 1902)
33	*Phlaeoba infumata* (Brunner, 1893)
34	*Phlaeoba panteli* (Bolivar, 1902)
35	*Schistocerca gregaria gregaria* (Forskal, 1775)
36	*Spathosternum prasiniferum prasiniferum* (Walker, 1871)
37	*Stenocatantops splendens* (Thunberg, 1815)
38	*Trilophidia annulata* (Thunberg, 1815)

6.2. Screening of experimental species (short-horned-grasshopper)

The study of correlation coefficient indicated that the population of *S. pr. prasiniferum* showed positive correlation with physical factors and population density (Table 4) and highly significant correlation between population of this species and relative humidity.

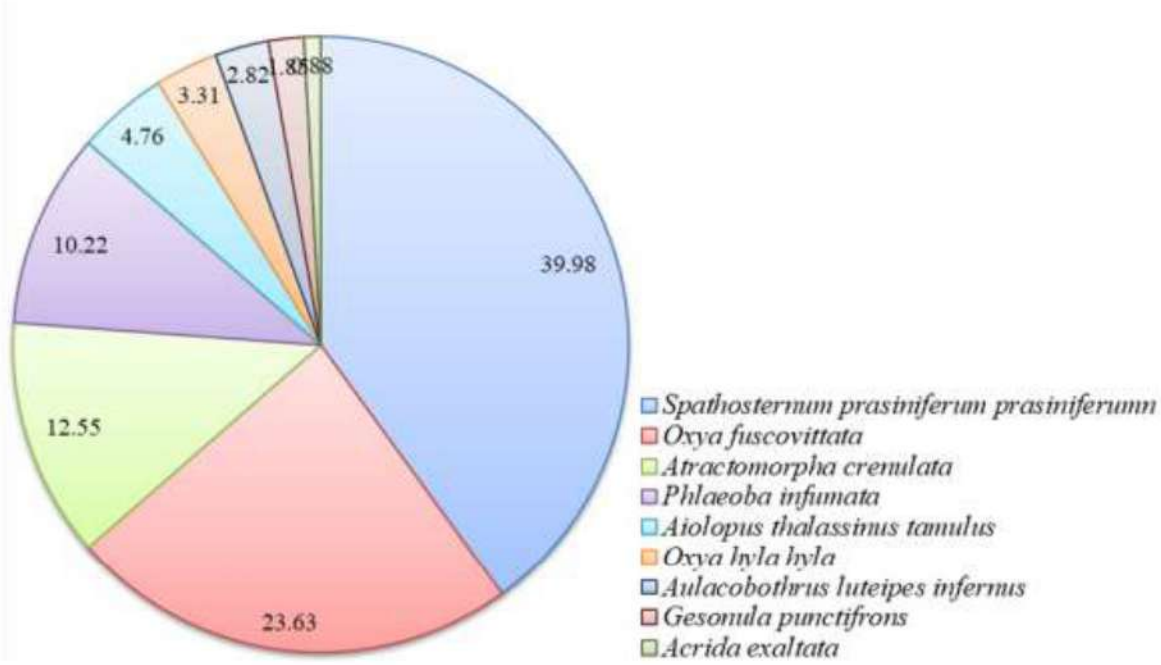

Fig. 4. Initial screening for experimental species of different agri-field species (So many species grossly studied).

Table 4. Percentage (%) of available acridid species.

Sl. No	Name of the species	Population density (%)
1	*Spathosternum prasiniferum prasiniferumn*	39.98
2	*Oxya fuscovittata*	23.63
3	*Atractomorpha crenulata*	12.55
4	*Phlaeoba infumata*	10.22
5	*Aiolopus thalassinus tamulus*	4.76
6	*Oxya hyla hyla*	3.31
7	*Aulacobothrus luteipes infernus*	2.82
8	*Gesonula punctifrons*	1.85
9	*Acrida exaltata*	0.88

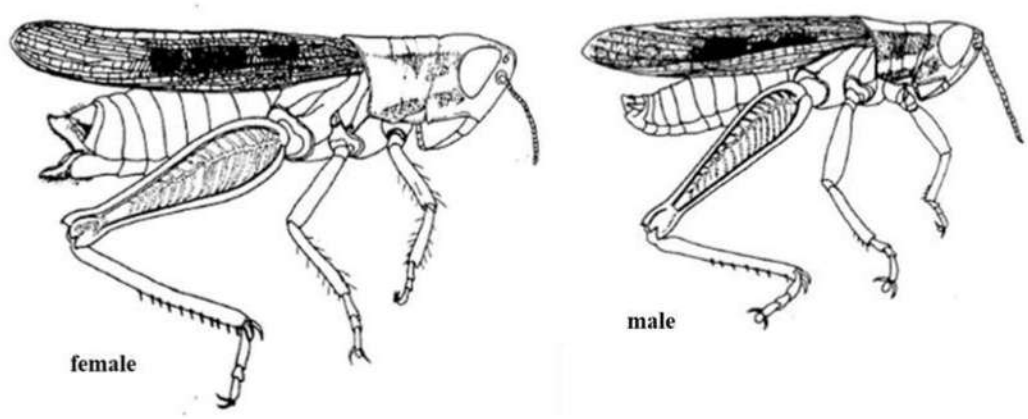

Plate 24. General morphology of *Spathosternum prasiniferum prasiniferum* (female and male).

6.2.1. Biology of the *Spathosternum prasiniferum prasiniferum* (Walker 1871)

Small, green, integument finely rugose almost smooth. Head conical, fastigium of vertex obtusely angular or parabolic, filiform antennae, frontal ridge which is narrow and sulcate, two broad band which is blackish or dark greenish running behind the lower part of the eyes and below the lateral carine of the pronotum which is banded above by a narrow pale yellow line and below by a broader one. Pronotum smooth, flattened, median and lateral carinae present. Presternal process large, strongly antero-posteriorly compressed, spatulate and inclined backwards and mesosternal interspace strongly constricted. Elytra and wing fully developed or shortened, tegmina is light brown towards the base and subhyaline beyond, central area with a longitudinal black streak, generally almost obsolete in the male and well-marked in female, wings hyaline, hind femur slender, arolium large. Male supra-anal plate is elongate angular, abdominal appendage of female is unusually short (Plate 24).

a. Female

Ovipositor short, moderately robust with curved valves. Supra-anal plate long triangular, apex obtuse with a median sulcus from base to nearly the top. Cercus conical, apex sub-

acute not reaching beyond the supra-anal plate. Sub-genital plate is longer than broad, hind margin with a small, median triangular projection (Plate 24).

Morphometry (mm): Length of body 17.9; length of antenna 4.8; length of pronotum 4.4; length of tegmen 14.4; length of hind femur 10.8; length of hind tibia 8.8.

b. Male

Supra-anal plate is elongated, triangular, apex obtuse, disc with median basal impression and in the middle with a transverse impression. Cercus simple, conical sometimes slightly incurved, not reaching beyond the supra-anal plate. Sub genital plate is short, sub-conical with obtuse apex. Epiphallus with undivided bridge is short anchorae and lobiform lophi (Plate 24).

Morphometry (mm): Length of body 14.4; length of antenna 3.9; Length of pronotum 3.4; length of tegnum 11.4; length of hind femur 8.6; length of hind tibia 6.8.

c. Distribution

India (West Bengal, Andhra Pradesh, Arunachal Pradesh, Bihar, Goa, Himachal Pradesh, Jammu and Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Rajasthan, Tamil Nadu); Myanmar; S.E. China; Thailand and Vietnam.

6.3. Toxicity studies on *Spathosternum prasiniferum prasiniferum* by widely used nimbecidine (azadirachtin)

6.3.1. General observation

In the present study, AZT was tested against *S. pr. prasiniferum* to study mortality, nutritional-indices, OS and antioxidant status of this insect. In present investigation, the percent mortality (%) of insects were observed after topical application of AZT (Table 7). Insects exposed to different doses of AZT showed certain behavioural changes such as abnormal behaviour of restlessness, sudden quick and jerky movements were observed at

low concentration of pesticide whereas, increased movements accompanied with swarming movements and loss of equilibrium followed by loss of body tonus and coordinated movements, sagging of legs followed by paralysis, loss of postural balance and complete immobilization. The animals tumbled down and rested on their dorsal side with their ventral side up. The animals remained in this state without any recovery until death and abnormal oviposition are observed in the grasshoppers exposed to higher concentrations of AZT. I watched insect's percent mortality (%) increases with the doses of AZT treatment in both sexes. Thus the results suggest that the insect is more susceptible to AZT. Percent mortality (%) of insect was dose and time dependently higher (Fig. 11.e). The mortality (%) within the control group is very low and no stress are observed on died control insects but sex and dose dependant mortality percentage (%) are observed after AZT toxicity (Fig. 5.a). The percent mortality (%) of the insects in different concentrations of AZT were plotted against their time-concentrations showed a typical sigmoid curve after 48-hrs (Fig. 5.a). It is noticed that, male is more sensitive to the AZT and female is more tolerant with AZT. The mortality (%) increases almost linearly and after the 5 ppm dose, mortality rate (%) becomes higher (Fig. 17.b). From the Fig. 11.a it can be suggested that the present experimental species and other similar species have high nymphal life span (≥80 days), but the present species has ~100 days' life span. But in Fig. 17.d it is clear that adult female has appreciably higher life span than its male counterpart and this is true for all the primarily studied organism from that habitat. This finding is in parity with our biochemical and molecular biological data; that is female has a greater adaptability in terms of OS and antioxidant activities.

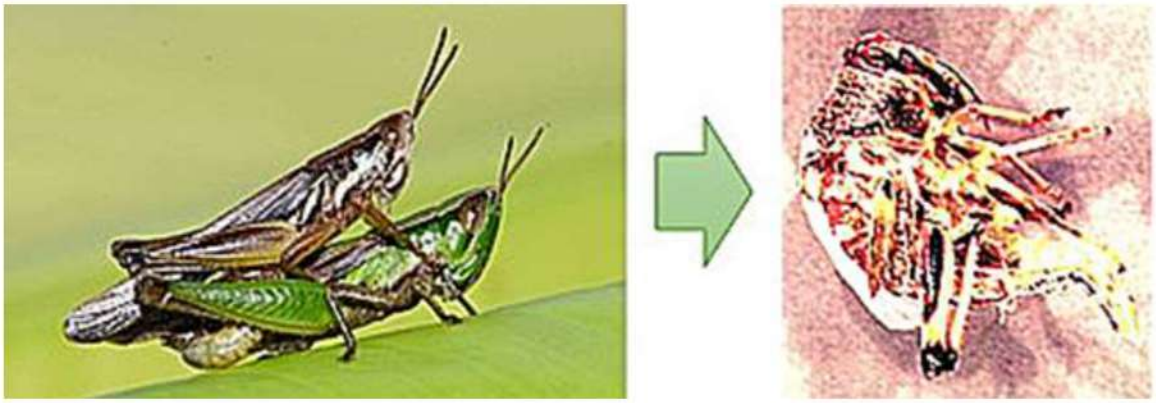

Plate 25. Deformity of *Spathosternum prasiniferum prasiniferum* after AZT toxicity.

Exposure of insects to different concentrations of AZT showed no mortality up to 1 ppm, 30% mortality at 5 ppm, 50% mortality at 10 ppm, 70% mortality at 15 ppm and 100% at 20 ppm (Fig. 11.e). The percent mortality (%) of the insects in different concentrations of AZT were plotted against their concentrations showed a typical sigmoid curve (Fig. 11.e). Thus, the results suggest that the insects are more susceptible to AZT.

6.3.2. Nutritional-indices

Nutritional-indices have been analysed in adult stages of *S. pr. prasiniferum* after dose dependant AZT toxicity (Fig. 5.b). Food ingestion, digestion and consumption index (CI) which are necessarily increased in higher doses, are statistically significant ($P<0.001$) during feeding and the approximate digestibility (AD) is not significant increased whereas efficiency of conversion of digested-food (ECD) and efficiency of conversion of ingested-food (ECI) are reduced after the AZT treatment (Fig. 5.b).

6.3.3. Oxidative stress parameters activities

Present results on gut protein, malondialdehyde (MDA), non-protein-soluble-thiols (NPSH), acetyl-cholinesterase (AChE) and alkaline-phosphatase (ALP) have been employed extensively for the one-way variance (ANOVA) studies to evaluate the significances of differences/dependence of different parameters (Table 12). It will elucidate the

influences on several biochemical and physiological processes on each other's functions (Fig. 6 and 7). Finally, the outcome of the interactive nature of these reactions determines the toxicological and the adaptive outcome of the AZT. This ANOVA study is performed separately on in-vivo and in-vitro of male and female insects. It is noticed that dependence between any two of the above-mentioned parameters is highly significant ($P<0.001$) (Fig. 6 and 7). When multiple comparison Dunnett's test (2-sided) is performed, it is noticed significant ($P<0.001$ to $P<0.01$) due to the dose dependant (periodic in several cases) response of AZT. Some of the individual dosage is found to be insignificant in both female and male gut, but in case of female changes are more obvious and significant (Fig. 6 and 7). The in-vivo and in-vitro both sexes gut protein contents have been significantly ($P<0.001$) different compared to control (Fig. 6.a and 7.a). A significant ($P<0.001$) increased levels of lipid peroxidation (LPO) are found in-vivo female and male gut (Fig. 6.b and 7.b) after AZT administration and an increased level of NPSH were found in-vivo and in-vitro both sexes gut cells (Fig. 6.c and 7.c). Azadirachtin dose dependently reduced ALP activity in-vivo gut cells of both sexes (Fig. 6.d). Acetyl-cholinesterase activity were significantly decreased in female whereas male represent higher enzymatic activity (Fig. 6.e and 7.d).

In-vivo brain protein from both sexes were significantly ($P<0.001$) increased whereas in-vitro brain from both sexes were almost unaffected among treated in comparison to controls insects (Fig. 12.a and Fig. 13.a) in response to AZT (Table 13). Haemolymph protein from both sexes were significantly ($P<0.001$) higher after dose dependant AZT toxicity in comparison to that of control (Fig. 14.a). Insects exposed to in-vivo AZT treatment showed higher LPOs in brains for both sexes (Fig. 12.b). Moreover, a significant ($P<0.001$) decrease in the levels of LPO were observed in only in-vitro male brain but increased in female brain (Fig. 13.b). An increased level of LPO were found in the

haemolymph of both sexes treated with AZT (Fig. 14.b) in compared to control. Increased NPSH were found in-vivo both sexes brain tissues after dose dependant AZT toxicity (Fig. 12.c). Moreover, a significant decreased NPSH levels were observed in only in-vitro male brain but increased in female brain (Fig. 13.c). Non-protein-soluble-thiol levels of haemolymph from both sexes after AZT toxicity were significantly increased (Fig. 14.c). Azadirachtin toxicity depressed in in-vivo female brain ALP activity but increased in-vivo male brain (Fig. 12.d) and decreased ALP activities in both sexes treated haemolymph in compare to that of control group (Fig. 14.d). Acetyl-cholinesterase activity significantly decreased in-vivo male brain and increased in-vivo female brain (Fig. 12.e) whereas, inhibition of AChE activities was noticed in-vitro brain tissues of both sexes after AZT treatment (Fig. 13.d). The AChE activity of haemolymph from both sexes was significantly depressed in AZT groups (Fig. 14.e).

In-vivo female gonad protein is significantly ($P<0.001$) and predominantly decreased but no significant change was noticed in case of male gonad protein (Fig. 18.a). Especially in female the protein concentration was found to be variable in relation to the AZT concentration (Table 14). No significant changes were noticed in in-vitro gonads from both sexes when compared to that of control (Fig. 19.a) except in female at 15 ppm dose where the protein was found to be approximately double than that of the control in response to dose dependant AZT application. In case of instar-II and instar-IV tissue protein is significantly ($P<0.001$, Table 14) increased in comparison to that of control (Fig. 21.a). In-vivo AZT treated gonads show higher MDA level for both sexes except in female at 15 ppm dose. In-vivo males the values reach the highest level of MDA, both at 1 ppm and at 20 ppm of AZT. And the levels were higher only in female in-vitro condition only at 15 ppm dose but not in all concentrations. The dose dependant changes are analysed by ANOVA (Table 14) which was found to be highly significant ($P<0.001$). Signif-

icant ($P<0.001$) increased MDA level were observed in-vivo female and moderately higher in male gonads (Fig. 18.b). In-vitro female gonad showed higher MDA after AZT treatment (Fig. 19.b). An increased level of MDA is found in both instar tissues (Fig. 19.b) in comparison to control. In case of instar-II, MDA increased at initial AZT concentration (0.013 pmole to 0.022 pmole) and at higher dose this group showed multiphasic responses. An increased level of NPSH is found in-vivo both sexes gonads after AZT treatment (Fig. 18.c). Moreover, a significant decreased NPSH level is observed in only instar-IV whereas the level increased in instar-II (Fig. 21.c). It is noticed that in-vitro treatment with AZT does not affect the level of NPSH in males and that in females it produces a polyphasic response between 10 and 20 ppm (Fig. 19.c). Azadirachtin toxicity significantly depresses ALP activity of in-vivo male gonad but increases in-vivo female gonad (Fig. 18.d). Acetyl-cholinesterase activity significantly increases in-vivo female and male goads (Fig. 18.e) whereas decreases activity in-vitro gonads of both sexes after dose dependant AZT toxicity in comparison to control (Fig. 19.d). The in-vitro experimental result suggests that AChE activity decreased from 0.24 to 0.2 nmole/mg protein/min from control to 10 ppm AZT dose and this value was 0.17 nmole/mg protein/min at 20 ppm dose. In female gonad it slightly increased from 5 to 10 ppm doses and drastically decreased at 15 ppm but came closer to the normal value at next higher dose. In-vitro male AChE activity decreased from 0.24 to 0.2 nmole/mg protein/min from control to 10 ppm AZT dose and this value was 0.17 nmole/mg protein/min at 20 ppm dose. In female gonad it slightly increased from 5 to 10 ppm doses and drastically decreased at 15 ppm but came closer to the control value at higher next dose (Fig. 19.d). The AChE activity of instar-II is significantly depressed (from 5 to 20 ppm) after AZT treatment than that of corresponding doses in instar-IV (Fig. 21.e). But when the dose effects are compared to the corresponding control then the instar-II is only seen to have increased with 1 ppm

AZT, but all other values are approx. The same, while the AChE activity of instar-IV increased with all the doses, being the highest at 10 ppm and that between 5 and 15 ppm a multiphasic behaviour was presented again. So, there was a recognizable increase in activities/status of evaluated OS-markers against currently used insecticide (Plate 29, 30 and 31).

6.3.4. Antioxidant enzyme activities

Dose dependent in-vivo and in-vitro gut SOD activity was significantly higher after AZT treatment of both sexes than that of controls and an increased CAT activity were observed only in female but decreased in male gut (Fig. 8).

Superoxide-dismutase activities significantly higher in in-vivo and in-vitro brain from both sexes were in response to AZT treatment (Fig. 15). Superoxide-dismutase activities of AZT treated male haemolymph were significantly higher whereas the significant ($P<0.001$) low level of SOD was recorded in female haemolymph compared to that of control (Fig. 15). The CAT activity in in-vivo brain of both sexes increased and that in-vitro male brain decreased with dose dependent manner (Fig. 15). Catalase activities increased in female haemolymph whereas male haemolymph showed gradual decrease with AZT doses (Fig. 15). In-gel GPx activity in in-vivo and in-vitro brain of both sexes were not significantly varied (Fig. 15) and haemolymph from female were not altered but increased trend about two fold in male in response to AZT toxicity in comparison to controls (Fig. 15).

Superoxide-dismutase activity in in-vivo gonads from both sexes after toxicity is significantly higher than that of control (Fig. 20) and showed decreased activity in in-vitro gonads. Superoxide-dismutase activity of both instar tissues are shown significantly higher level in comparison to control (Fig. 22). In-vivo gonads of both sexes CAT activities increases and in-vitro male gonads CAT activities decreases gradually with the in-

crease of dosage (Fig. 20). In in-vitro, the activity in males increased significantly to 5 ppm AZT and then fell to near the control value at 20 ppm of AZT dose. Catalase activity increased instar-IV whereas instar-II showed moderate change with the increase of dosage in comparison to control (Fig. 22). In detail, instar-II (except with 1 ppm AZT) there seems to be no significant differences. In-gel GPx activity in in-vivo and in-vitro brain (Fig. 20) of both sexes and also in juvenile tissues (Fig. 22) is not significantly varied in response to AZT toxicity in comparison to controls.

Densitometry data of band strength of all enzymatic activities were performed in gonad tissues of both sex (Fig. 20) and in nymphal tissues (Fig. 22). The densitometry data of in-vivo and in-vitro investigation suggest dose dependant increase is higher in female in case of all enzymes except in-vivo GPx activity. We did not find appreciable GPx band in in-vitro condition. In case of in-vitro condition enzyme was almost unresponsive to AZT in case of male gonad. In case of nymphal tissues AZT dose dependant enzymatic activity change was only noticed in case of CAT of instar-IV tissue. In case of amylase and SOD activity irregular increase was noticed in only instar-II tissue.

6.3.5. Nutrient-metabolizing-enzyme activities

Increased amylase activity was observed in female gut whereas inhibition effect was observed in male gut at increased concentration of AZT and decreased activity of cellulase was observed in female gut whereas increased activity is observed in male gut (Fig. 9). In-vivo and in-vitro female brain showed increased amylase activity whereas moderately activity decreased in male brain of both experimental groups with the increasing AZT doses (Fig. 15). No inhibition of starch hydrolysing activity are found in haemolymph of both sexes (Fig. 15). In-vivo and in-vitro female gonads showed increased amylase activity whereas decreased in in-vivo and in-vitro male gonads with the increase of dosage in

comparison to control (Fig. 20). Inhibition of starch hydrolysing activity is found in in-star-IV and increased activity is found in instar-II (Fig. 22).

6.2.6. Genotoxicity

Gel electrophoresis was conducted to examine any kind of DNA damage following AZT exposure. The DNA-fragmentation were observed into consideration to understand extent of DNA damage in different treatment categories. Present work indicates that, OS-induced gut of both sexes DNA-laddering are observed in comparison to control (Fig. 10). The DNA stability was found to be decreased in in-vivo and in-vitro brain of both sexes in compared with control (Fig. 16).

In-vivo and in-vitro gonads of both sexes and DNA-laddering of in-vitro juvenile tissues induced by AZT doses are observed in comparison with control (Fig. 23). Differential responses were noticed in case of AZT dependant DNA-laddering pattern. In case of nymph more DNA-laddering was noticed in instar-II versus instar-IV.

Table 5. Comparative study of fecundity, fertility, egg mortality (%), nymphal mortality (%) and preparation of life table of selected acridid species in semi-laboratory condition (data represent the means, n=20).

Sl. No	Species	Fecundity	Fertility	Egg Mortality (%)	Nymphal Mortality (%)	Adult life span (Days)/ Individuals	
						Male	Female
1	*Acrida exaltata*	71.75	68.00	5.22	41.17	18.75	29.50
2	*Aiolopus thalassinus tamulus*	55.50	45.75	17.56	78.14	12.75	21.50
3	*Atractomorpha crenulata*	118.5	105.0	11.39	69.55	19.25	30.00
4	*Aulacobothrus luteipes infernus*	15.75	14.50	7.930	74.13	14.50	20.50
5	*Gasonula punctiforns*	9.80	8.40	14.28	45.45	13.25	27.75
6	*Oxya fuscovittata*	30.75	27.25	11.38	45.87	17.50	34.25
7	*Oxya hyla hyla*	27.75	24.00	13.51	45.83	26.75	41.50
8	*Phlaeoba infumata*	55.75	52.75	5.380	43.60	26.25	33.00
9	*Spathosternum prasiniferum prasiniferum*	11.25	8.75	22.22	45.71	22.00	32.50

Table 6. Comparative study of nymphal and adult rearing period of selected acridid species in semi-laboratory condition (data represent the means, n=20).

Sl. No.	Species name	Individual No		Mean no of eggs/pod	Egg Mortality (%)	Mean no of Eggs hatched	Nymphal Mortality (%)	No of individual produced/ generation		Adult Wet Weight (mg)		Sex Ratio	
		M	F					M	F	M	F	M	F
1	*Acrida exaltata*	1	1	71.75	5.22	68	41.17	15	25	435.26	575.26	5	3
2	*Aiolopus thalassinus tamulus*	1	1	55.5	17.56	45.75	78.14	4	6	181.73	226.1	5	3
3	*Atractomorpha crenulata*	1	1	118.5	11.39	105	69.55	13.25	19.25	99.47	257.89	5	3
4	*Aulacobothrus luteipes infernus*	1	1	15.75	7.93	14.5	74.13	1.5	2.25	58.42	85.26	5	3
5	*Gasonula punctiforms*	1	1	9.8	14.28	8.4	45.45	1.6	2	80.52	141.05	1	1
6	*Oxya fuscovittata*	1	1	30.75	11.38	27.25	45.87	6	8.75	214.73	247.89	5	2
7	*Oxya hyla hyla*	1	1	27.75	13.51	24	45.83	5	8	124.73	179.47	2	2
8	*Phlaeoba infumata*	1	1	55.75	5.38	52.75	43.6	13.25	16.5	96.31	165.78	3	1
9	*Spathosternum prasiniferum prasiniferum*	1	1	11.25	22.22	8.75	45.71	2	2.75	59.73	74.73	2	1

Table 7. Relative toxicity of different doses of AZT on *S. pr. prasiniferum*. End point: Lethal concentration to kill 50% of test species. (P<0.001, significantly different from control group, 10 animals in each group, ANOVA analysis at df 5).

Does	24-hrs		48-hrs		72-hrs	
	Female	Male	Female	Male	Female	Male
Control	0±0	0±0	0±0	0±0	3.72826±0.343429	0±0
1 ppm	0±0	3.7659±0.420564	3.982333±0.654569	11.16667±1.258306	10.678±0.651807	15.9146±1.090558
5 ppm	0±0	10.06713±0.227843	6.566367±0.486369	13.929±0.765444	30.19637±0.573351	50.04607±0.298776
10 ppm	3.506237±0.394655	20.4089±0.801788	39.84333±0.734638	46.47933±0.779079	56.08039±1.234737	72.66787±0.576438
15 ppm	10.07717±0.175751	36.5454±0.422655	56.14927±0.488196	73.18651±0.790332	83.17077±0.755091	96.783±0.704738
20 ppm	20.16913±0.642324	43.33579±0.333317	53.2146±0.82226	69.96184±0.178737	93.67427±0.380117	99.85549±0.253313

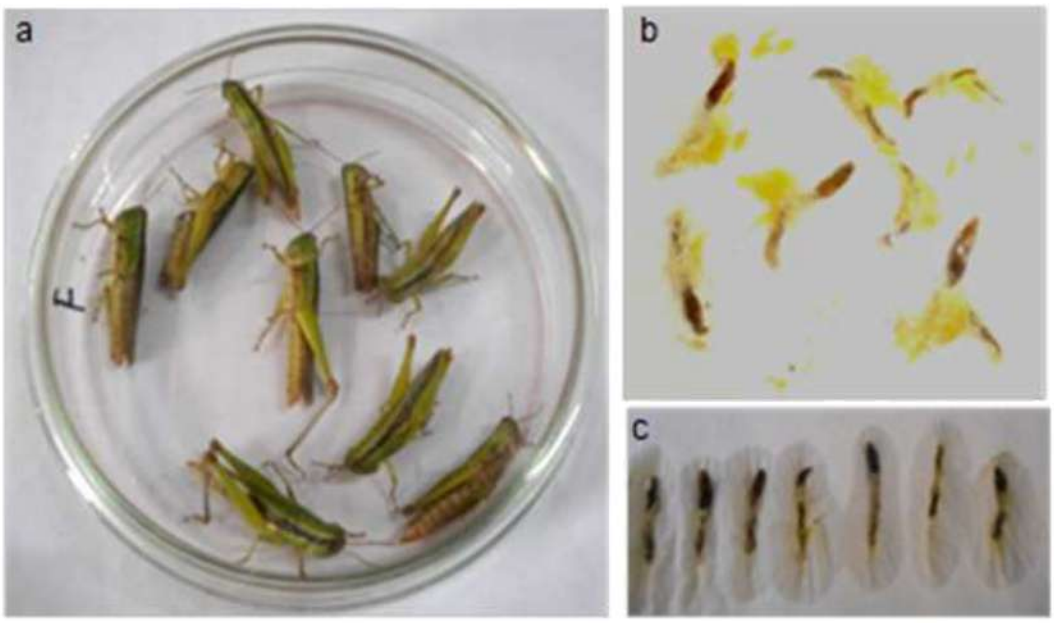

Plate 26. Dissected organs after in-vivo and for in-vitro treatment. **(a)** Treated female and male *S. pr. prasiniferum*. **(b)** Dissected whole gut tissue after in-vivo treatment. **(c)** Dissected whole gut tissue for in-vitro treatment.

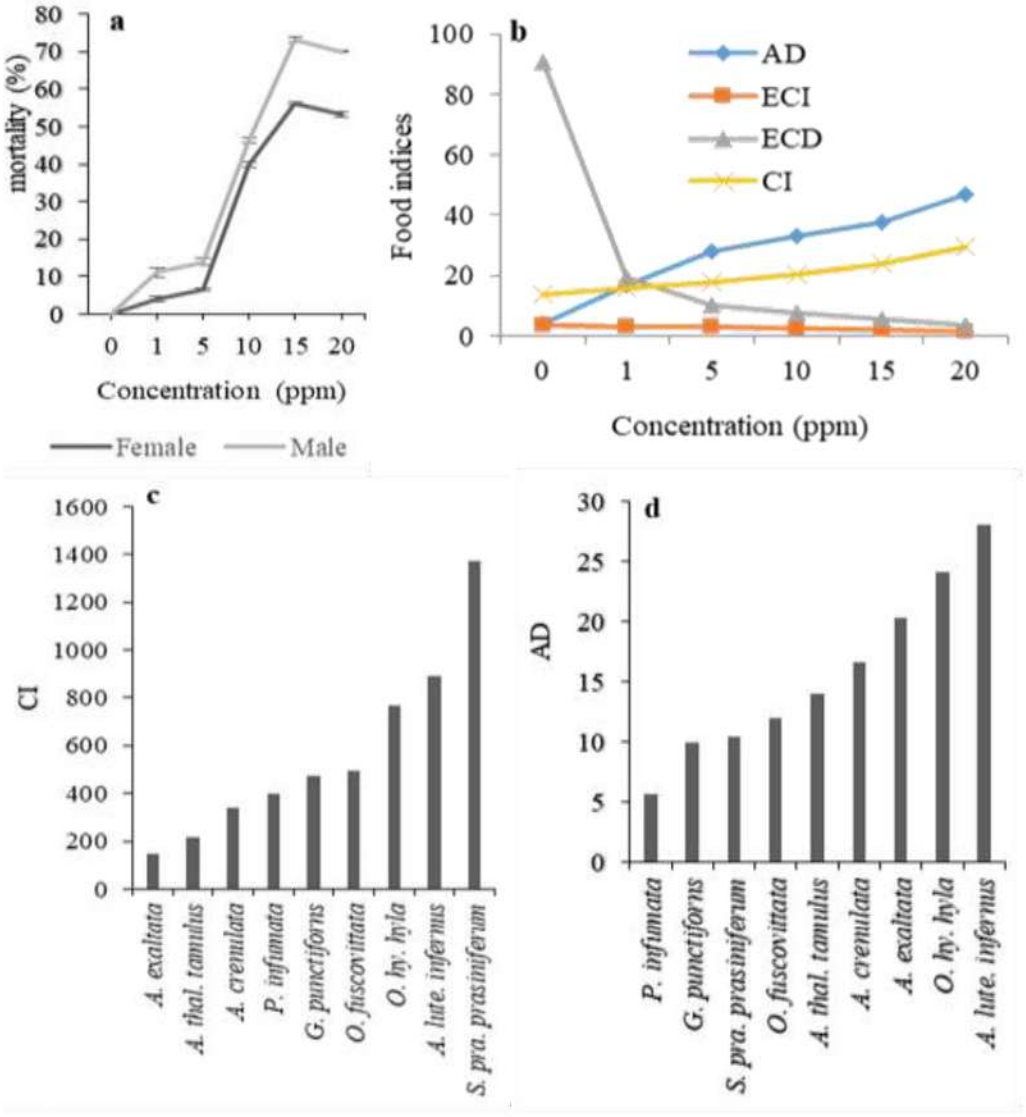

Fig. 5. (a) Sex dependant percent mortality (%) after AZT treatment with different concentrations in *S. pr. prasiniferum* (data represent the means ± SD, n=20; control vs. treated groups). **(b)** Nutritional-indices after AZT treatment with different concentrations in *S. pr. prasiniferum* (data represent the means ± SD of triplicate analysis) means within lines are significantly different (P<0.001, ANOVA; control vs. treated groups). **(c)** Consumption index (CI) of some common acridids under semi-laboratory condition (data represent the means, n=20). **(d)** Approximate digestibility (AD) of some selected acridids under semi-laboratory condition (data represent the means, n=20).

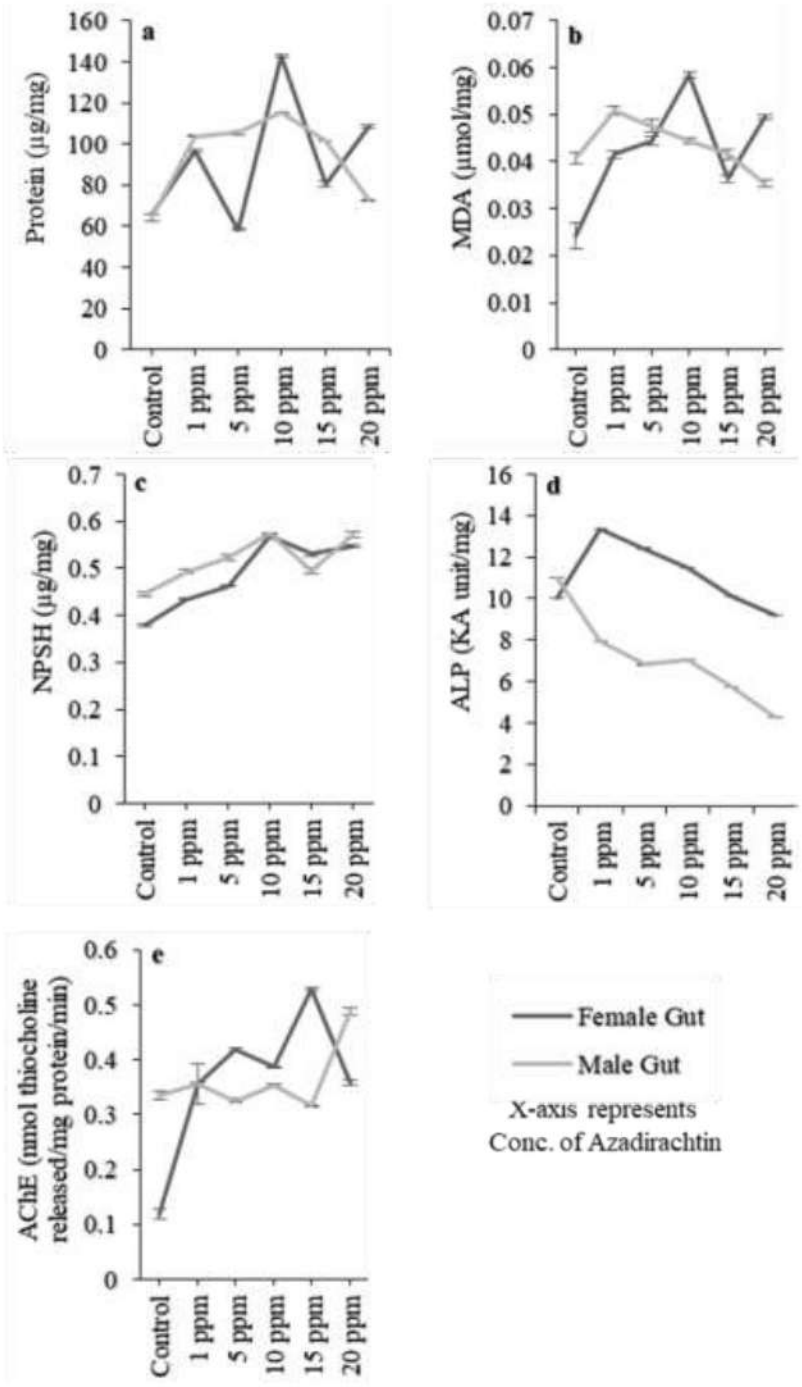

Fig. 6. Total protein **(a)**, MDA **(b)**, NPSH **(c)**, ALP **(d)** and AChE **(e)** content of in-vivo gut after 48-hrs AZT treatment with different concentrations in *S. pr. prasiniferum* (data represent the means ± SE of triplicate analysis) means within lines are significantly different ($P<0.001$, ANOVA).

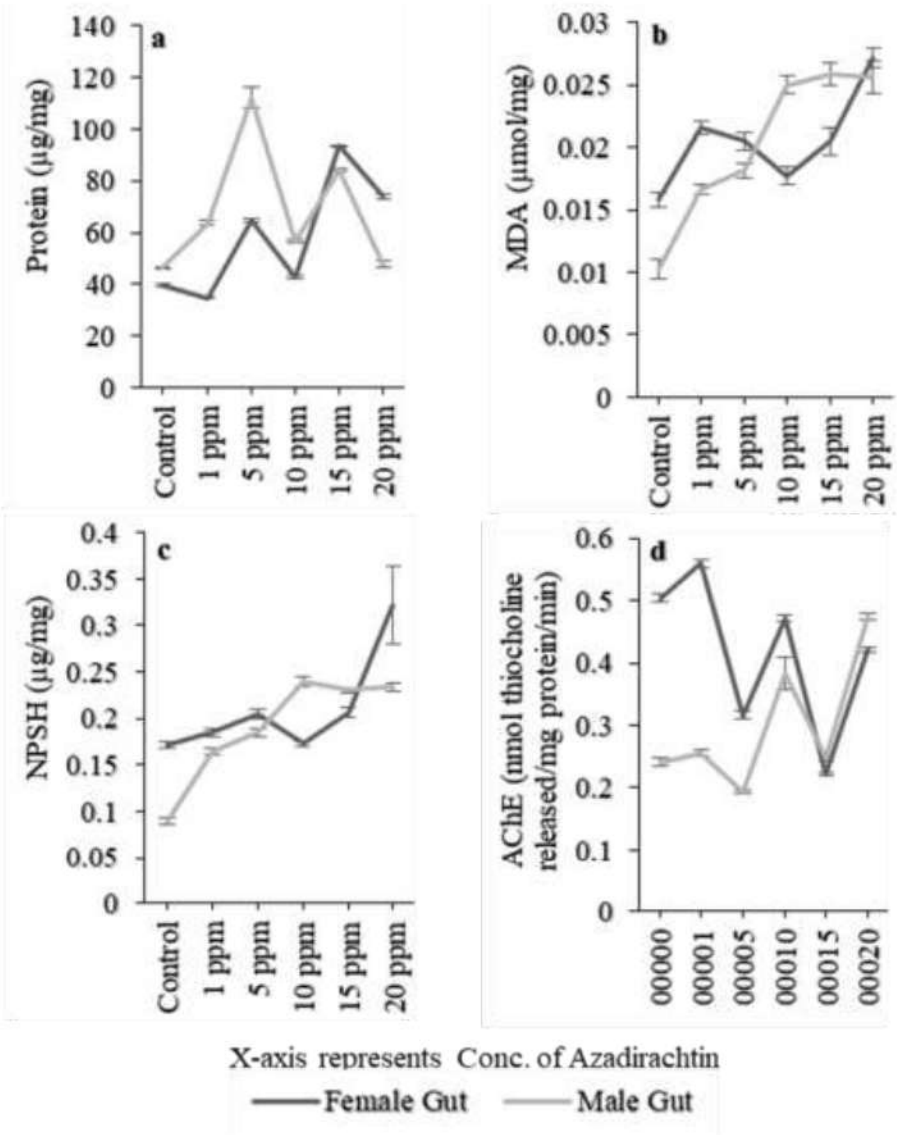

Fig. 7. Total protein **(a)**, MDA **(b)**, NPSH **(c)** and AChE **(d)** content of in-vitro gut after 6-hrs AZT treatment with different concentrations in *S. pr. prasiniferum* (data represent the means ± SE of triplicate analysis) means within lines are significantly different ($P<0.001$, ANOVA).

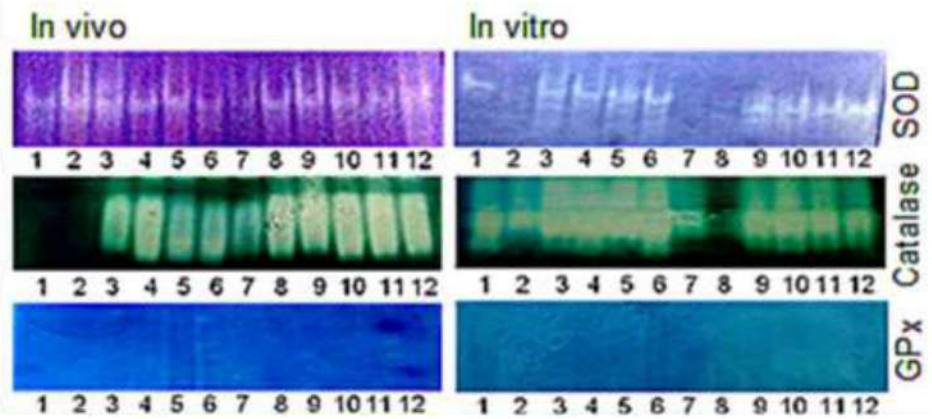

Fig. 8. Effect of AZT on specific activity of marker enzymes of gut in *S. pr. prasiniferum* showing on a polyacrylamide gel (both in-vivo and in-vitro experiment)- Lane distribution of each panel: Lane 1-6 female tissue (1- control, 2- 1 ppm, 3- 5 ppm, 4- 10 ppm, 5- 15 ppm, 6- 20 ppm) and Lane 7-12 male tissue (7- control, 8-1 ppm, 9- 5 ppm, 10- 10 ppm, 11- 15 ppm, 12- 20 ppm); panel **a**- SOD, panel **b**- CAT and panel **c**- GPx.

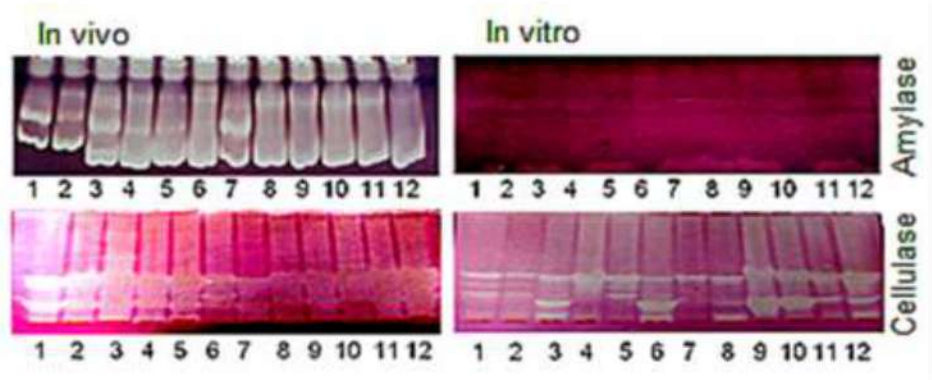

Fig. 9. Effect of AZT on specific activity of digestive enzymes of gut in *S. pr. prasiniferum* on a polyacrylamide gel (both in-vivo and in-vitro experiment)- Lane distribution of each panel: Lane 1-6 female tissue (1- control, 2- 1 ppm, 3- 5 ppm, 4- 10 ppm, 5- 15 ppm, 6- 20 ppm) and Lane 7-12 male tissue (7- control, 8- 1 ppm, 9- 5 ppm, 10- 10 ppm, 11- 15 ppm, 12- 20 ppm); panel **a**- amylase and panel **b**- cellulase.

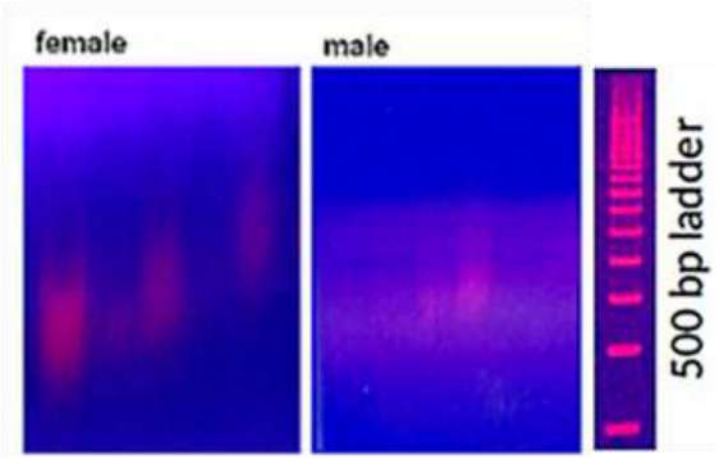

Fig. 10. Effect of AZT on gut DNA of *S. pr. prasiniferum*. Lane distribution of each panel: Lane 1-6 (1- control, 2- 1 ppm, 3- 5 ppm, 4- 10 ppm, 5- 15 ppm, 6- 20 ppm).

Table 8. Nutritional-indices (CI, AD, ECD and ECI) of common acridid species studied in semi-laboratory condition (data represent the means, n=20).

Sl. No	Species	Food Consumption /individual (mg)	Faecal matter production (mg)	Mean Weight (mg)	Duration of feeding period (Days)	Consumption Index (CI)	Approximate Digestibility (AD)	Efficiency of Conversion of Digested food (ECD)	Efficiency of Conversion of Ingested food (ECI)
1	*Acrida exaltata*	2524.74	2011.61	516.25	29.5	144.27	20.32	399.08	8.76
2	*Aiolopus thalassinus tamulus*	2073.08	1783.17	207.5	21.5	214.80	13.98	257.42	8.81
3	*Atractomorpha crenulata*	2042.47	1702.34	181.25	30	338.06	16.65	106.65	2.64
4	*Aulacobothrus luteipes infernus*	3427.95	2466.51	78.75	20.5	892.35	28.04	86.41	1.22
5	*Gasonula punctiforms*	1832.27	1650.69	107.5	27.75	472.92	9.90	75.09	3.07
6	*Oxya fuscovittata*	3336.30	2935.80	230	34.25	496.81	12.00	113.58	1.44
7	*Oxya hyla hyla*	2682.60	2035.15	145	41.5	767.78	24.13	129.13	2.61
8	*Phlaeoba infumata*	1926.22	1818.17	158.75	33	400.4	5.60	69.83	1.72
9	*Spathosternum prasiniferum prasiniferum*	2800.18	2508.42	66.25	32.5	1373.67	10.41	142.84	0.57

Table 9. Instar wise growth of common acridid species studied in semi-laboratory condition (data represent the means, n=20).

Sl. No	Species	1st instar	2nd instar	3rd instar	4th instar	5th instar	6th instar	7th instar
1	*Acrida exaltata*	61.15	80	131.25	27.5	125.62	80.62	
2	*Aiolopus thalassinus tamulus*	13.71	25	36.25	27.5	25.62	71.87	
3	*Atractomorpha crenulata*	37.03	10	21.25	46.25	36.25	18.75	
4	*Aulacobothrus luteipes infernus*	8.68	6.25	13.75	7.5	7.5	12.5	13.75
5	*Gasonula punctiforns*	14.23	10	2.5	27.5	25	22.5	
6	*Oxya fuscovittata*	32.96	17.5	33.75	20	75.62	14.37	28.75
7	*Oxya hyla hyla*	16.71	22.5	25	20	25.62	20.62	37.5
8	*Phlaeoba infumata*	14.96	22.5	36.25	27.5	38.12	11.87	
9	*Spathosternum prasiniferum prasiniferum*	14.07	1.25	11.25	8.75	8.12	5.62	10

Table 10. Live biomass of common acridid species from nearby agri-field of West Bengal, India (data represent the means, n=20).

Sl. No	Species name	Biomass after one generation (mg)		Total biomass/ generation (a+b)
		M (a)	F (b)	(mg)
1	*Acrida exaltata*	6528.95	14381.6	20910.5
2	*Aiolopus thalassinus tamulus*	726.94	1356.63	2083.58
3	*Atractomorpha crenulata*	1318.03	4964.47	6282.5
4	*Aulacobothrus luteipes infernus*	87.6316	191.84	279.47
5	*Gasonula punctiforns*	128.84	282.1	410.947
6	*Oxya fuscovittata*	1288.42	2169.08	3457.5
7	*Oxya hylahyla*	623.68	1435.79	2059.47
8	*Phlaeoba infumata*	1276.18	2735.53	4011.71
9	*Spathosternum prasiniferum prasiniferum*	119.47	205.52	325

Table 11. Nutrient contents of common edible acridid species studied in semi-laboratory condition (data represent the means, n=20).

Sl. No	Species name	Male Protein (%)	Female Protein (%)	Male Carbohydrate (%)	Female Carbohydrate (%)
1	*Acrida exaltata*	61.5± 2.12	52.5± 9.19	2± 1.41	2.5± 0.70
2	*Aiolopus thalassinus tamulus*	54± 1.41	51.5± 0.70	6.5± 0.70	3± 1.41
3	*Atractomorpha crenulata*	54± 2.82	50.5± 4.94	4± 1.41	4.5± 0.70
4	*Aulacobothrus luteipes infernus*	50.5± 3.53	49± 2.82	3.5± 0.70	2.5± 0.70
5	*Gasonula punctiforns*	55.5± 0.7	52.5± 0.70	2.5± 0.70	1.5± 0.70
6	*Oxya fuscovittata*	61.5± 2.12	58.5± 2.12	6± 1.41	4± 1.41
7	*Oxya hyla hyla*	59.5± 3.53	59.5± 2.12	5.5± 0.70	3.5± 0.70
8	*Phlaeoba infumata*	49.5± 3.53	45± 4.24	2.5± 0.70	3± 1.41
9	*Spathosternum prasiniferum prasiniferum*	63.5± 2.12	63± 1.41	5± 1.41	3.5± 0.70

Table 12. Summary statistics for quantity biochemical variables of gut in *S. pr. prasiniferum* after AZT toxicity (P<0.001, significantly different from control group, 10 animals in each group, ANOVA analysis at df 5)

In vivo	F	P	In vitro	F	P
female			**female**		
Gut-Protein	1692.025	.000	Gut-Protein	1560.169	.000
Gut-MDA	76.000	.000	Gut-MDA	24.894	.000
Gut-NPSH	462.444	.000	Gut-NPSH	10.422	.000
Gut-ALP	26775.782	.000	Gut-AChE	488.850	.000
Gut-AChE	72.802	.000			
male			**male**		
Gut-Protein	482.278	.000	Gut-Protein	191.866	.000
Gut-MDA	29.547	.000	Gut-MDA	57.167	.000
Gut-NPSH	89.850	.000	Gut-NPSH	206.874	.000
Gut-ALP	26126.022	.000	Gut-AChE	86.667	.000
Gut-AChE	161.937	.000			

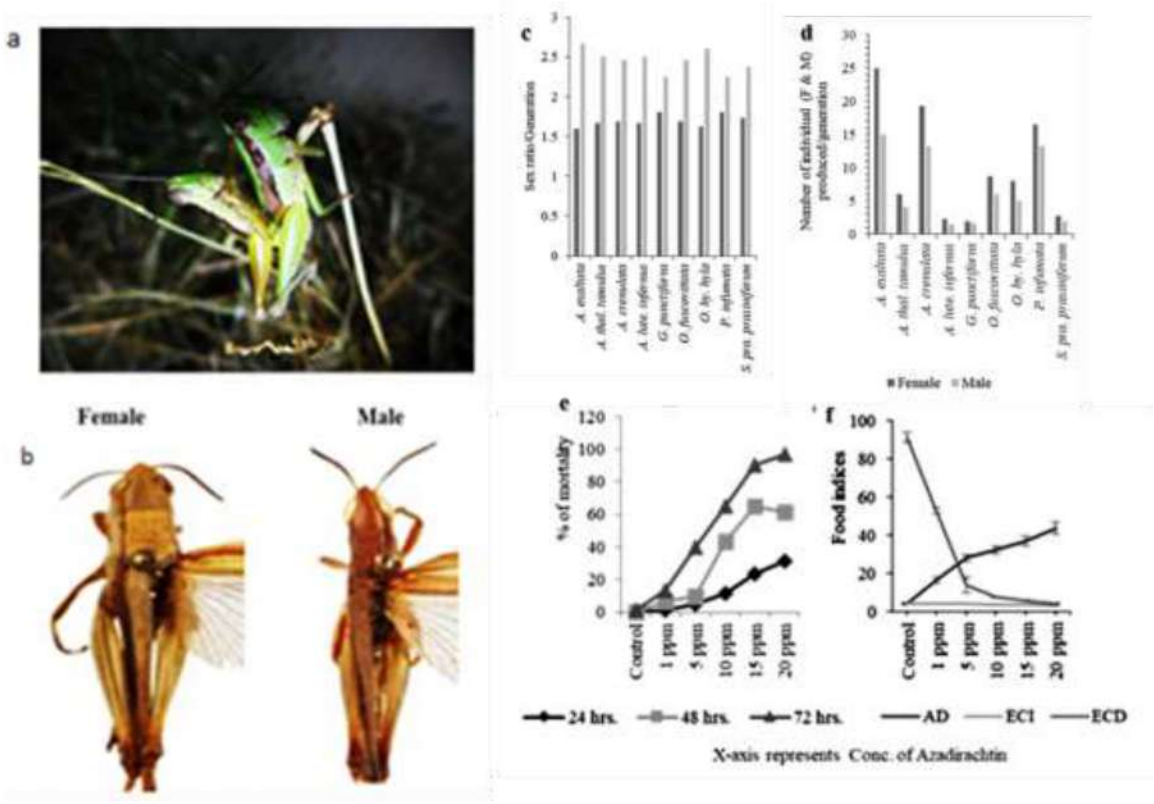

Fig. 11. Left panel: **(a)** and **(b)** *Spathosternum prasiniferum prasiniferum* (Walker 1871). Right panel: (**c)** Sex ratios per generation of available acridid species from West Bengal, India. **(d)** Reproduction rate of selected acridid species. **(e)** Cumulative percent mortality (%) after AZT treatment (data represent the means ± SD, n=20). **(f)** Nutritional-indices after AZT treatment (data represent the means ± SD of triplicate analysis) means within lines are significantly different ($P<0.001$, ANOVA).

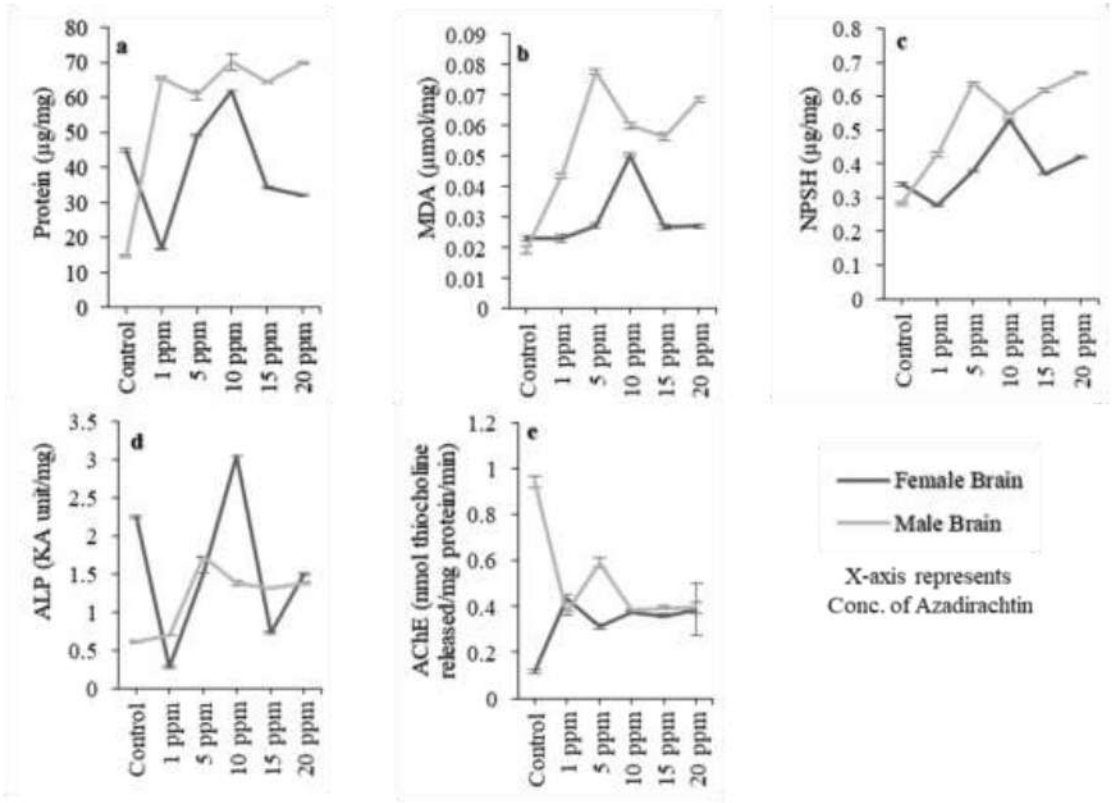

Fig. 12. Total protein **(a)**, MDA **(b)**, NPSH **(c)**, ALP **(d)** and AChE **(e)** contents of in-vivo brain after AZT treatment with different concentrations in *S. pr. prasiniferum* (data represent the means ± SE of triplicate analysis) means within lines are significantly different ($P<0.001$, ANOVA).

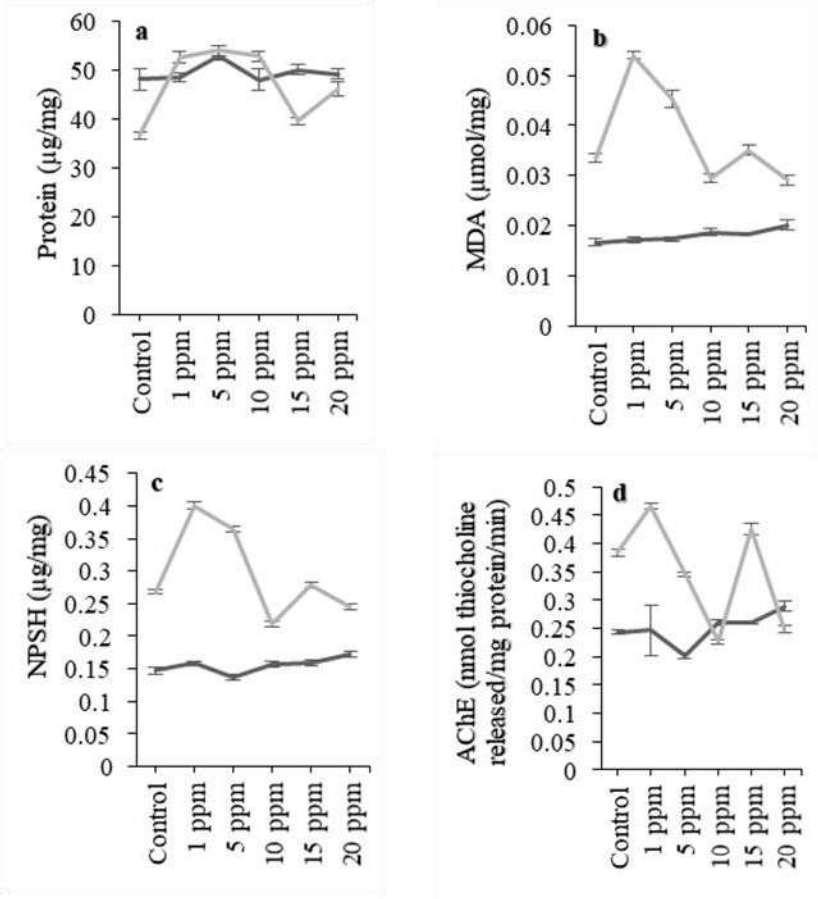

Fig. 13. Total protein **(a)**, MDA **(b)**, NPSH **(c)** and AChE **(d)** content of in-vitro brain after AZT treatment with different concentrations in *S. pr. prasiniferum* (data represent the means ± SE of triplicate analysis) means within lines are significantly different ($P<0.001$, ANOVA).

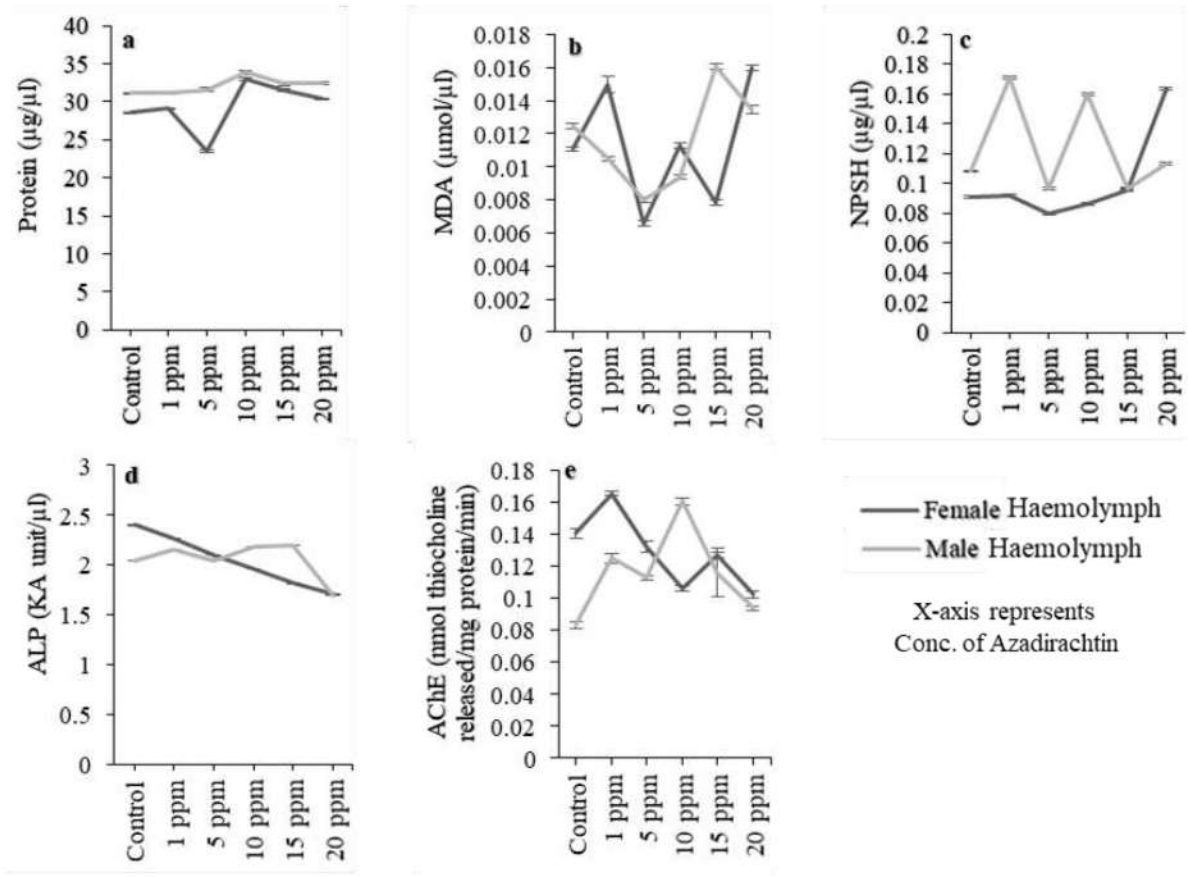

Fig. 14. Total protein **(a)**, MDA **(b)**, NPSH **(c)**, ALP **(d)** and AChE **(e)** content of haemolymph after AZT treatment with different concentrations in *S. pr. prasiniferum* (data represent the means ± SE of triplicate analysis) means within lines are significantly different ($P<0.001$, ANOVA).

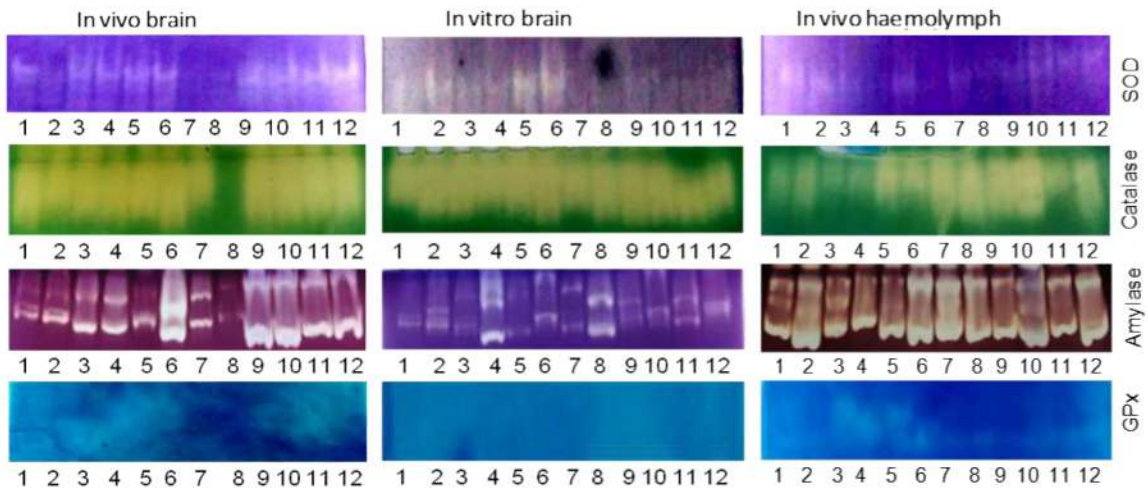

Fig. 15. Effect of AZT on specific activity of marker enzymes on brain and haemolymph of *S. pr. prasiniferum* showing on a polyacrylamide gel- Lane distribution of each panel: Lane 1-6 female tissue (1- control, 2- 1 ppm, 3- 5 ppm, 4- 10 ppm, 5- 15 ppm, 6- 20 ppm) and Lane 7-12 male tissue (7- control, 8- 1 ppm, 9- 5 ppm, 10- 10 ppm, 11- 15 ppm, 12- 20 ppm); panel **a**- SOD, panel **b**- CAT, panel **c**- amylase and panel **d**- GPx.

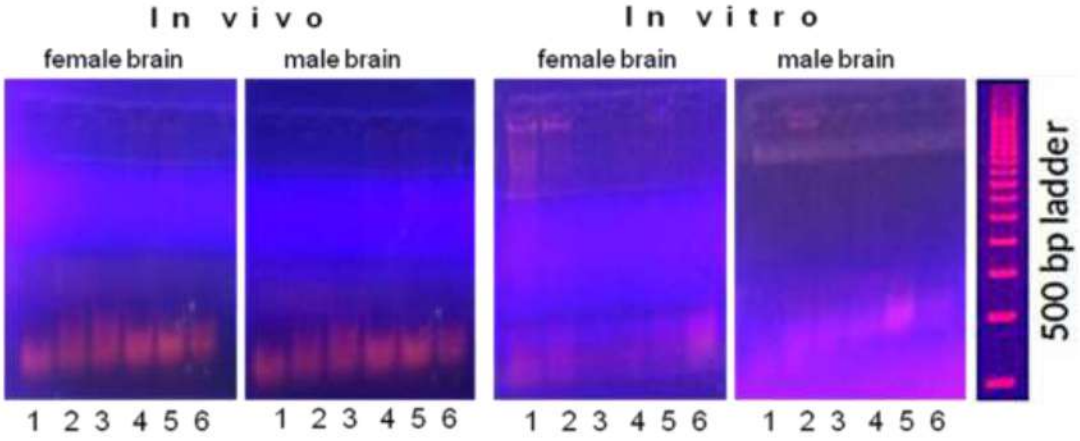

Fig. 16. Effect of AZT on neural DNA of *S. pr. prasiniferum*- Lane distribution of each panel: Lane 1-6 (1- control, 2- 1 ppm, 3- 5 ppm, 4- 10 ppm, 5- 15 ppm, 6- 20 ppm of AZT).

Table 13. Summary statistics for quantity biochemical variables brain and haemolymph in *S. pr. prasiniferum* after AZT toxicity (P<0.001, significantly different from control group, 10 animals in each group, ANOVA analysis at df 5).

In vivo female	F	P	In vivo male	F	P	In vitro female	F	P
Brain-Protein	2711.215	.000	Brain-Protein	394.099	.000	Brain-Protein	1.624	.228
Brain-MDA	145.544	.000	Brain-MDA	457.248	.000	Brain-MDA	4.078	.021
Brain-NPSH	405.059	.000	Brain-NPSH	945.160	.000	Brain-NPSH	7.127	.003
Brain-AChE	5.642	.007	Brain-AChE	150.850	.000	Brain-AChE	2.355	.104
Brain-ALP	5829.368	.000	Brain-ALP	692.781	.000	**In vitro male**		
Hemolymph-Protein	438.797	.000	Hemolymph-Protein	25.581	.000	Brain-Protein	47.323	.000
Hemolymph-MDA	245.028	.000	Hemolymph-MDA	280.182	.000	Brain-MDA	90.389	.000
Hemolymph-NPSH	1513.440	.000	Hemolymph-NPSH	1373.509	.000	Brain-NPSH	223.561	.000
Hemolymph-ALP	10207.130	.000	Hemolymph-ALP	5224.813	.000	Brain-AChE	221.245	.000
Hemolymph-AChE	96.230	.000	Hemolymph-AChE	17.100	.000			

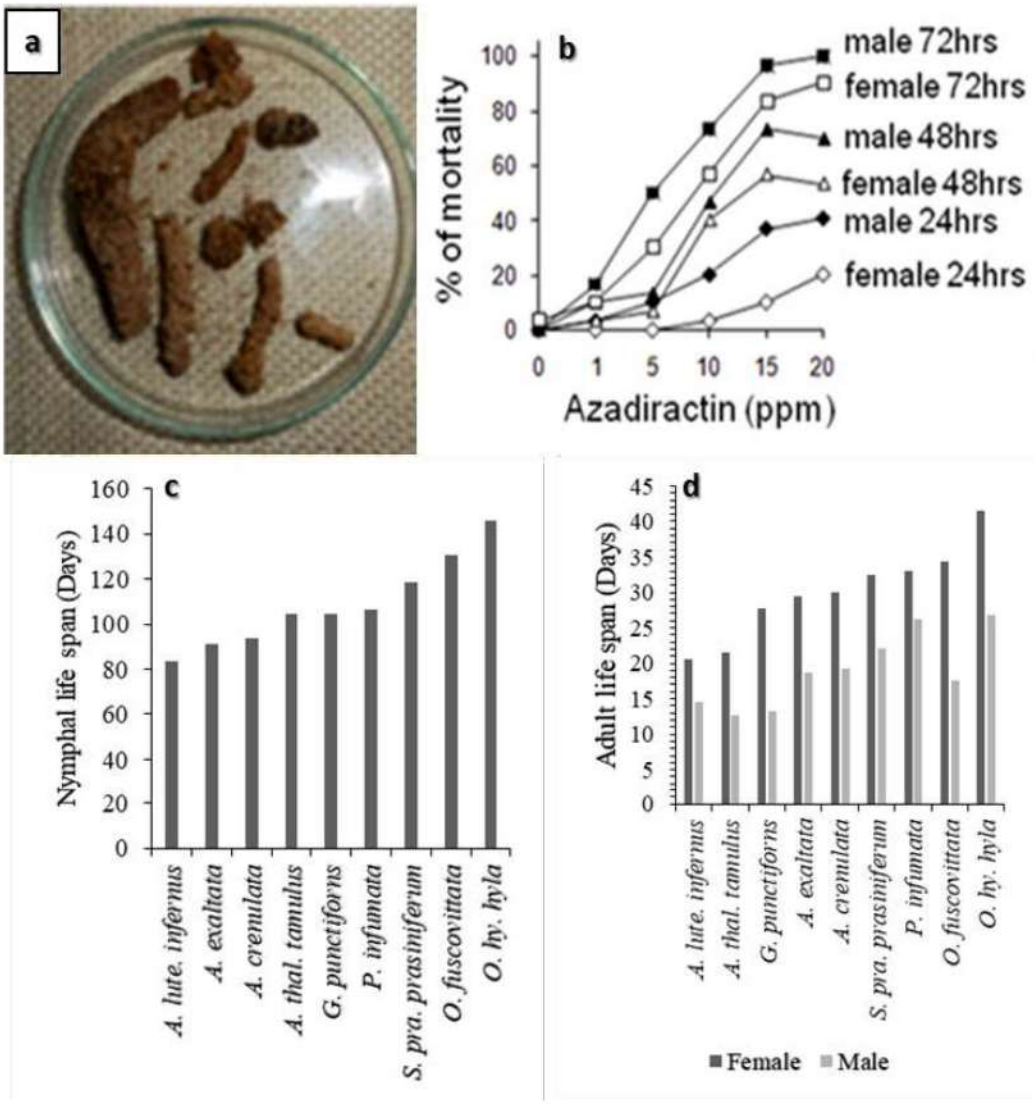

Fig. 17. (a) Egg pod laid by selected acridid species under semi-laboratory condition. **(b)** Dose, time and sex dependent percent mortality (%) (data represent the means, n=20; control vs. treated groups). **(c)** Nymphal life span of selected acridid species. **(d)** Adult life span of selected acridid species under semi-laboratory condition.

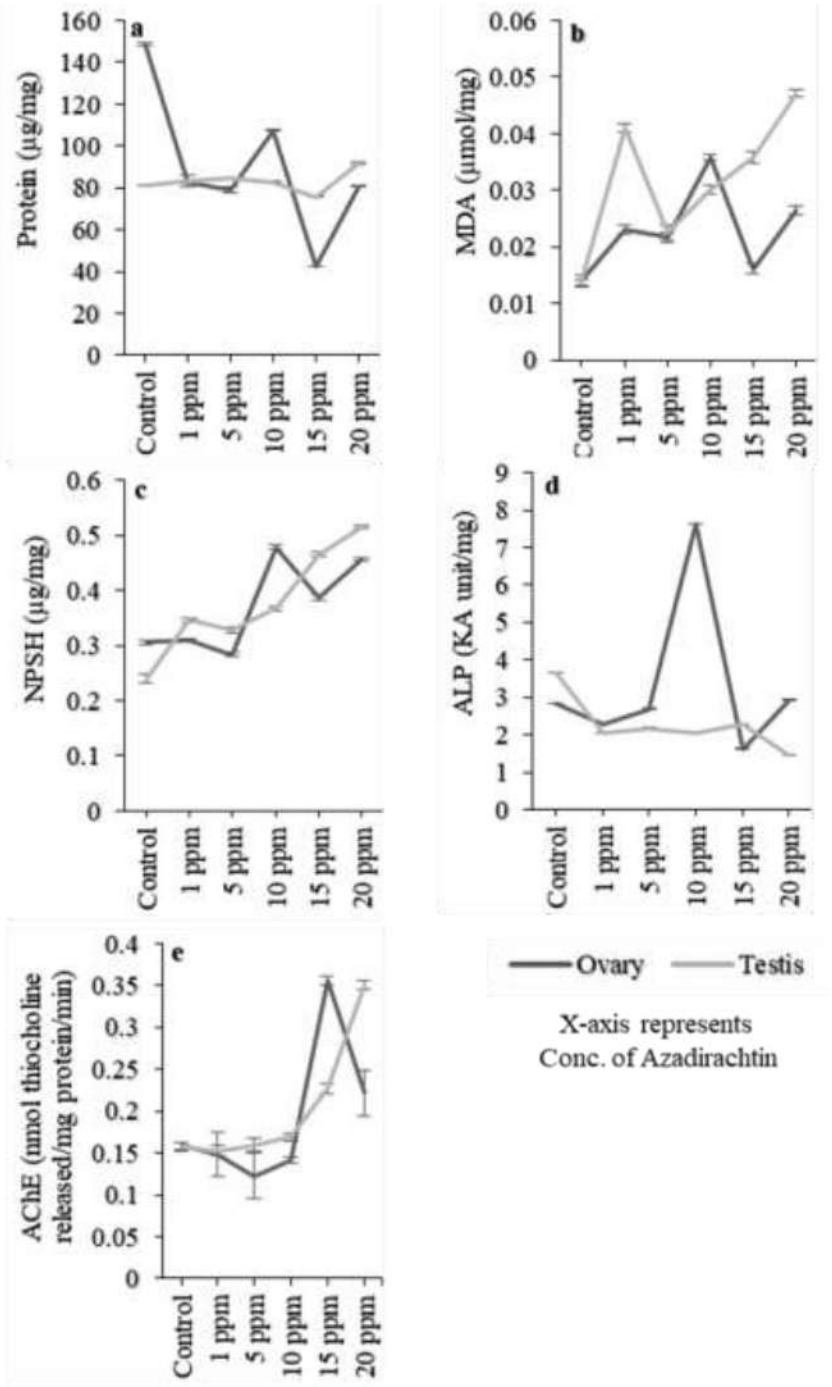

Fig. 18. Total protein **(a)**, MDA **(b)**, NPSH **(c)**, ALP **(d)** and AChE **(e)** content of in-vivo gonads after AZT treatment with different concentrations in *S. pr. prasiniferum* (data represent the means ± SE of triplicate analysis) means within lines are significantly different ($P<0.001$, ANOVA).

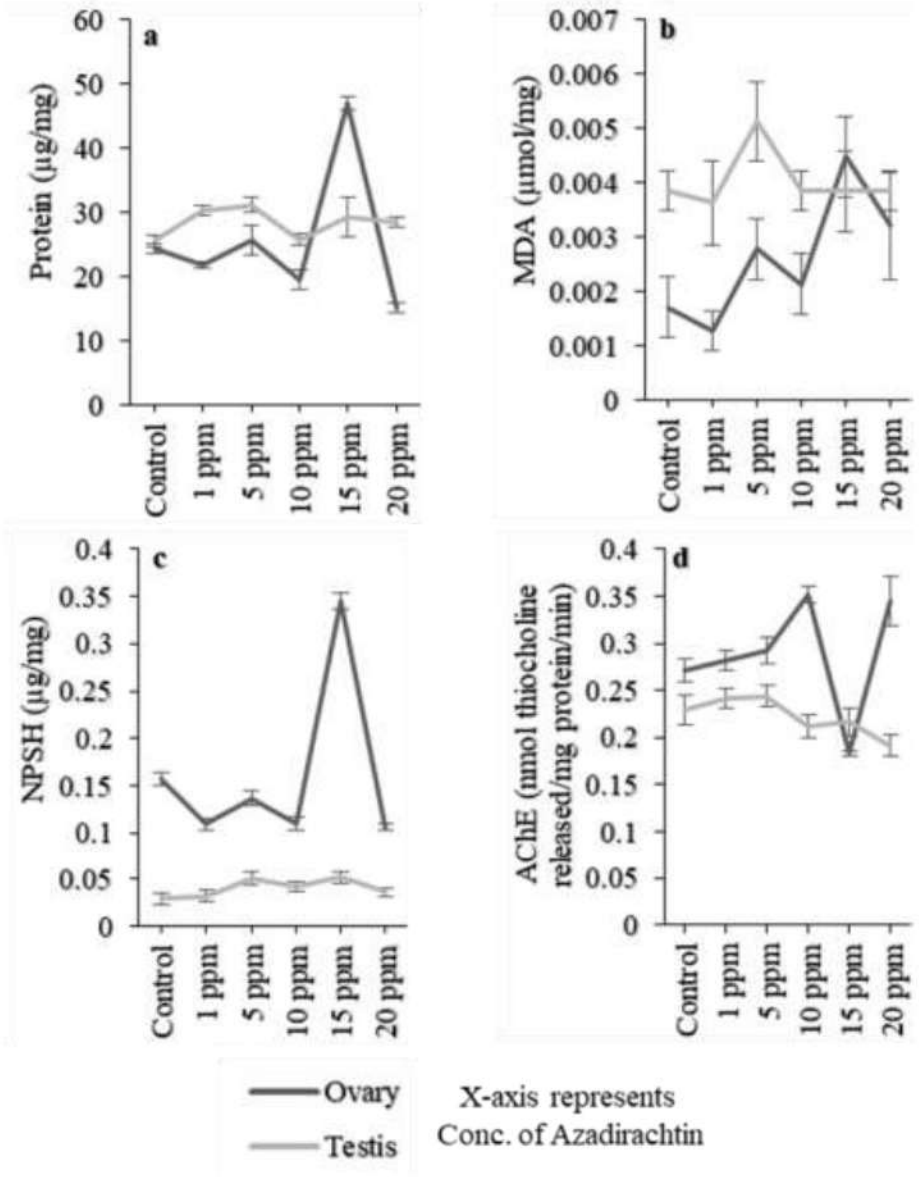

Fig. 19. Total protein **(a)**, MDA **(b)**, NPSH **(c)** and AChE **(d)** content of in-vitro gonads after AZT treatment with different concentrations in *S. pr. prasiniferum* (data represent the means ± SE of triplicate analysis) means within lines are significantly different ($P<0.001$, ANOVA).

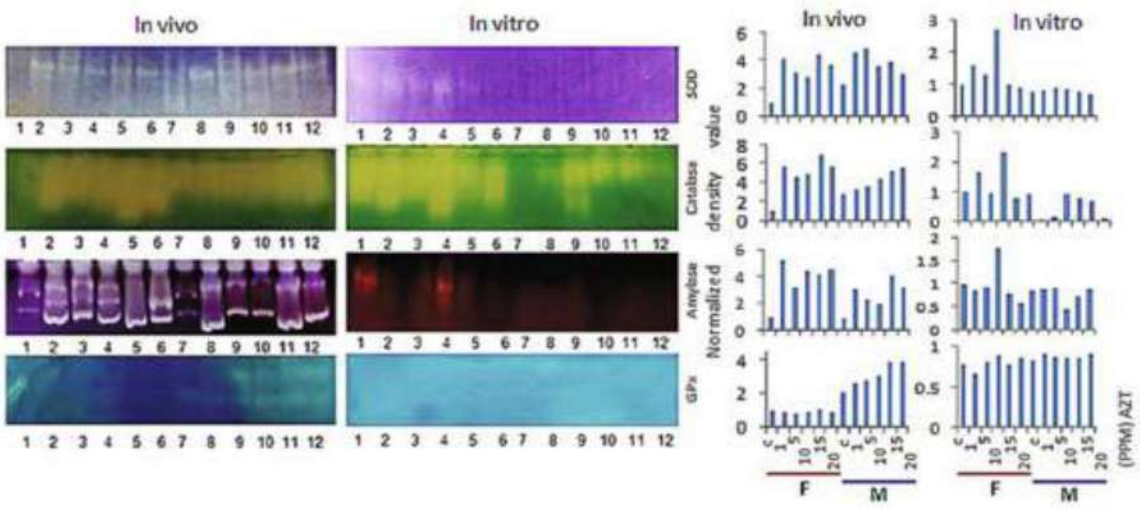

Fig. 20. Effect of AZT on specific activity of marker enzymes of gonad in *S. pr. prasiniferum* showing on a polyacrylamide gel (both in-vivo and in-vitro experiment)- Lane distribution of each panel: Lane 1-6 female gonad (1- control, 2- 1 ppm, 3- 5 ppm, 4- 10 ppm, 5- 15 ppm, 6- 20 ppm) and Lane 7-12 male gonad (7- control, 8- 1 ppm, 9- 5 ppm, 10- 10 ppm, 11- 15 ppm, 12- 20 ppm). panel **a**- SOD, panel **b**- CAT, panel **c**- amylase and panel **d**- GPx (The density of all the in-gel enzymatic activity bands were evaluated by the ImageJ software and the numerical data are plotted as bar-diagram to compare AZT effects with comparison to that of control).

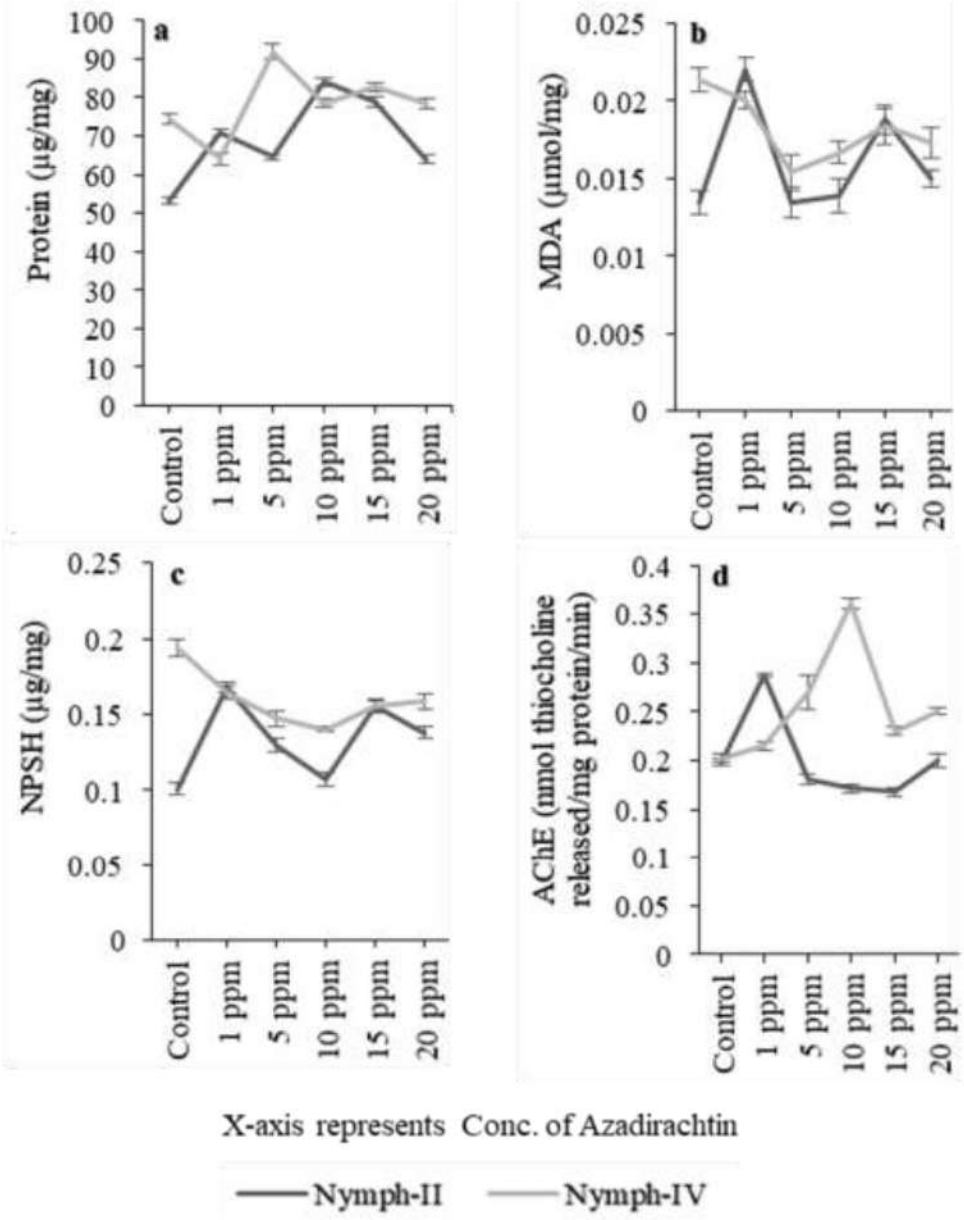

Fig. 21. Total protein **(a)**, MDA **(b)**, NPSH **(c)** and AChE **(d)** content of in-vitro juvenile tissue after AZT treatment with different concentrations in *S. pr. prasiniferum* (data represent the means ± SE of triplicate analysis) means within lines are significantly different ($P<0.001$, ANOVA).

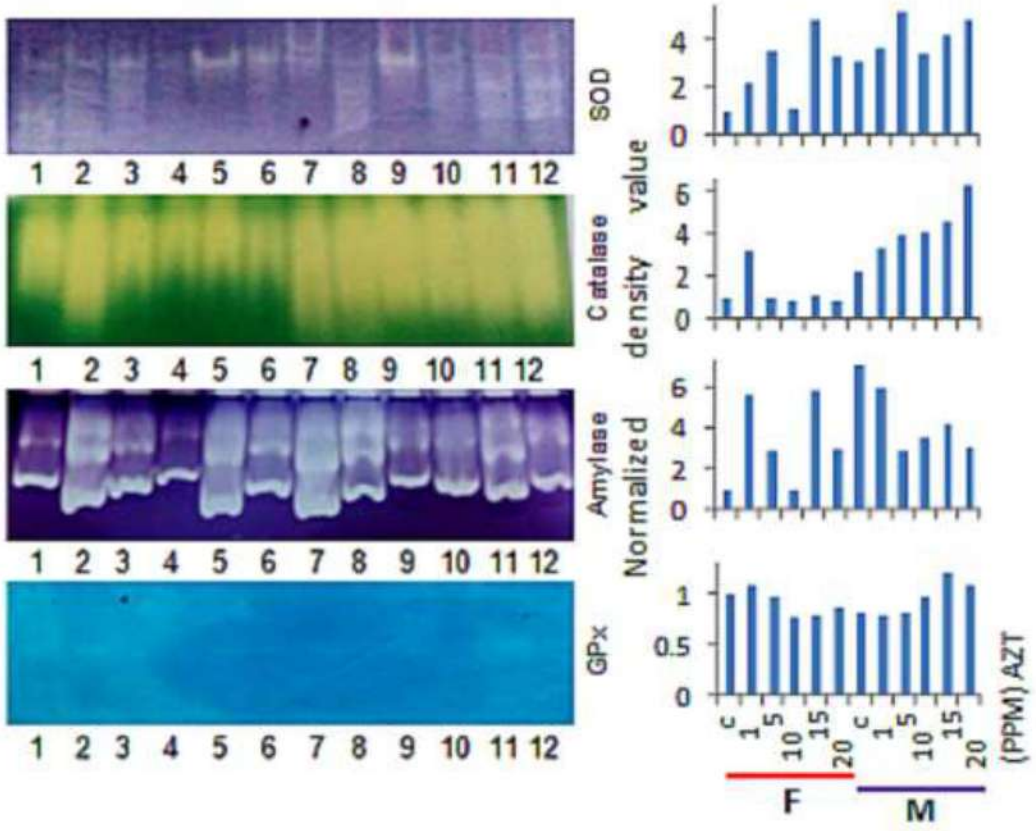

Fig. 22. Effect of AZT on specific activity of marker enzymes of juvenile tissue in *S. pr. prasiniferum* showing on a polyacrylamide gel (in-vitro experiment)- Lane distribution of each panel: Lane 1-6 instar-II tissue (1- control, 2- 1 ppm, 3- 5 ppm, 4- 10 ppm, 5- 15 ppm, 6- 20 ppm) and Lane 7-12 instar-IV tissue (7- control, 8- 1 ppm, 9- 5 ppm, 10- 10 ppm, 11- 15 ppm, 12- 20 ppm). panel **a**- SOD, panel **b**- CAT, panel **c**- amylase and panel **d**- GPx (The density of all the in-gel enzymatic activity bands were evaluated by the ImageJ software and the numerical data are plotted as bar-diagram to compare AZT effects with comparison to that of control).

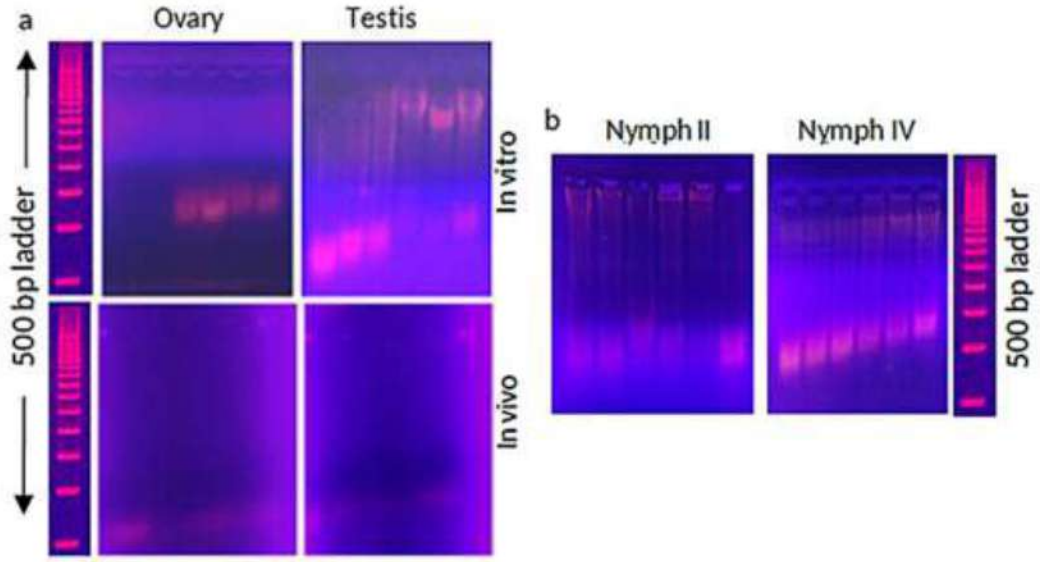

Fig. 23. Effect of AZT on gonadal DNA and juvenile tissue DNA of *S. pr. prasiniferum*-Lane distribution of each panel: Lane 1-6 (1- control, 2- 1 ppm, 3- 5 ppm, 4- 10 ppm, 5- 15 ppm, 6- 20 ppm).

Table 14. Summary statistics for quantity biochemical variables of gonads and juvenile tissues in *S. pr. prasiniferum* after AZT toxicity (P<0.001, significantly different from control group, 10 animals in each group, ANOVA analysis at df 5).

In vivo	F	P	In vitro	F	P	In vitro	F	P
female			**female**			Nymph-II-Protein	111.618	.000
Ovary-Protein	2624.345	.000	Ovary-Protein	69.954	.000	Nymph-II-MDA	16.891	.000
Ovary-MDA	89.031	.000	Ovary-MDA	3.091	.051	Nymph-II-NPSH	47.355	.000
Ovary-NPSH	462.802	.000	Ovary-NPSH	175.317	.000	Nymph-II-AChE	96.281	.000
Ovary-ALP	28348.160	.000	Ovary-AChE	18.147	.000	Nymph-IV-Protein	40.860	.000
Ovary-AChE	20.430	.000				Nymph-IV-MDA	6.009	.005
male			**male**			Nymph-IV-NPSH	15.535	.000
Testis-Protein	18.745	.000	Testis-Protein	2.288	.111	Nymph-IV-AChE	48.942	.000
Testis-MDA	171.919	.000	Testis-MDA	0.857	.537			
Testis-NPSH	438.852	.000	Testis-NPSH	2.845	.064			
Testis-ALP	4561.863	.000	Testis-AChE	2.449	.095			
Testis-AChE	184.389	.000						