Resistance to deltamethrin and fenitrothion in dubas bug, *Ommatissus lybicus* de Bergevin (Homoptera: Tropiduchidae) and possible biochemical mechanisms

Rashad Rasool Khan1,2*, Thuwaini Hashil Abdullah Al-Ghafri1, Salim Ali Humaid Al-Khatri1, Ibtisam Salim Suliman Al-Mazidi1 & Fatma Gharib Al-Rawahi1

Environmental pollution, ill-effects on human health, insecticide resistance development and insect pest resurgence are some serious problems that may arise due to excessive chemical spraying for pest control. Despite of heavy aerial and surface insecticide spraying, incomplete control of *Ommatissus lybicus* de Bergevin 1930 (Homoptera: Tropiduchidae) is reported in Oman every year, which requires investigation of insecticides resistance in pest. Fifteen populations of *O. lybicus*, collected from diverse vicinities were exposed along with a deltamethrin-selected (DEL-SEL) and lab-susceptible (LAB-SUS) strain to deltamethrin and fenitrothion insecticides in bioassay tests for estimation of their resistance status. All the field populations of *O. lybicus*, exhibited minor (RR = 3–5-folds) to low (RR = 5–10-folds) levels of resistance to deltamethrin, however, two out fifteen populations collected from Al-Hajir and Sint were found susceptible against fenitrothion (RR < 3-folds). Enzyme assays were conducted to detect the activities of cytochrome p-450-reductase (CPR), glutathione s-transferase (GST) and acetylcholinesterase (AChE) in the field collected, DEL-SEL and LAB-SUS strains of *O. lybicus*. Results revealed significantly increased activities of all enzymes in the field collected as well as DEL-SEL strains of *O. lybicus* when compared with LAB-SUS strains.

Over reliance on chemicals for insect control can result in insecticide resistance development in crop pests and human disease vectors. Excessive and overuse of chemical pesticides by the farming community as well as pest exterminators may give rise to certain problems especially when pesticide resistance is developed in insect pests. Regardless of insecticide resistance development, the over and repeated use of pesticides may result in pest resurgence, environmental pollution, side effects on non-target organisms and health risks to consumers14-2. Alternate pest management tactics, judicious usage of insecticides, synthesis and development of biorational chemicals and insecticide resistance management programs can serve as the most appropriate solution to all these issues6.

Dubas bug, *Ommatissus lybicus* de Bergevin 1930 (Homoptera: Tropiduchidae), is demonstrated as the serious pest of date palm in Oman and causes economic losses by reducing the production of quality date fruits7-11. The pest records are also reported from Saudi Arabia, Egypt, Iraq, Israel, Yemen, Pakistan, Qatar, United Arab Emirates (UAE) other than Oman15-18. The higher *O. lybicus* populations can damage the date palms to greater extent and can make 25–50% crop losses18. Scientists describing the impacts of climate change on infestation of *O. lybicus* has declared northern Oman at the edge of risk and stated that it will bear sever pest influxes in future owing to ecological aptness7.

---

1Plant Protection Research Center, Directorate General of Agriculture and Livestock Research, Ministry of Agriculture and Fisheries, Muscat 121, Oman. 2Department of Entomology, University of Agriculture, Faisalabad 38000, Pakistan. *email: rashadkhan@uaf.edu.pk
The plant protection research center in the Ministry of Agriculture and Fisheries, Oman applies insecticides including pyrethroids and organophosphates in alone and mixture combinations, through aerial and ground applications annually to regulate this obnoxious insect pest. A quantity of 523 tons of pesticides, costing 23 million dollars was sprayed for the control of this pest during 1993 to 2010. Regardless of this heavy spraying, incomplete control of the pest has been reported by the farmers every year. The repeated and heavy spraying of date palm orchards with pesticides have resulted in resistance development in O. lybicus16–18. Since, O. lybicus adults are not able to fly longer distance, hence, the intensive application of pesticides can result in evolution of resistance in such populations19. Laboratory and field reports on toxicities and efficiencies of insecticides including neonicotinoids, organophosphates, pyrethroids and botanicals etc., against O. lybicus are published by various authors18,20–27. Fenithrothion, diazinon, cypermetrin and deltamethrin were reported more efficient and toxic against O. lybicus under laboratory and field situations25,28. A susceptibility survey of deltamethrin and fenithrothion against field collected populations of O. lybicus was conducted in Oman and the authors suggested the use of synergist (piperonyl butoxide, PBO) along with the two pesticides for efficient pest control and mitigating the resistance development in the pest19. If the resistance in such instance is checked effectively, the pesticide will remain effective for pest management.

The underlying mechanisms of insecticide resistance have attained significant consideration and became popular subject of research interest for many researchers around the globe29,30. In order to identify and study the resistance development mechanisms in certain insects, enzyme assays have been widely used as tools, because they are simple and rapid to perform, and sensitive to obtain results even with small insect samples30. The literature reports and proves that amplified levels of mixed function oxidases, aid in resistance development to car-bamates, pyrethroids, organochlorines and organophosphates in certain insect pests31–37. Reports also describe the involvement of intensified esterases in mechanisms of resistance development against organophosphates, carbamates and pyrethroids38,39. Involvement and association of CPR, GST and AChE in the underlying mechanisms of resistance development against certain insecticides including organophosphates, organochlorines and pyrethroids has also been widely investigated and reviewed30,36,41–44. Furthermore, the AChE activities and role of insensitive and altered AChE in resistance to organophosphates and carbamates have been described in previous studies30,32,33,45.

As explained above, regardless of heavy insecticide spraying, severe infestations of O. lybicus are observed in date palm orchards in Oman. Development of resistance thus, has gained significant consideration as a reason for its control failures. No studies are documented to describe the underlying mechanisms of resistance development in O. lybicus against most commonly used insecticides. The present study was therefore planned to identify the underlying mechanism of resistance development in O. lybicus. Bioassay experiments were performed to appraise the status of insecticide resistance in different geographically isolated field collected O. lybicus populations against deltamethrin and fenithrothion (most often used insecticides against this pest in Oman). The enzyme analyses were further performed to detect the activities of CPR, GST and AChE in these field collected, DEL-SEL and LAB-SUS strains of O. lybicus. The findings reported in this study will provide baseline evidence about the current resistance status of the pest and the biochemical basis as a mechanism of resistance development for devising an effective and useful management strategy.

Results

Reference line susceptibility. The mortality scores of the laboratory reared nymphs of O. lybicus LAB-SUS strain were used as reference values for estimation of resistance and the corresponding LD50 values were used to calculate the resistance ratios (RRs) in the field collected populations. Both insecticides (deltamethrin and fenithrothion) showed highest toxicities to the LAB-SUS reference strain by displaying lowest LD50 values of 0.108 and 0.009 ppm, respectively. In comparison with the reference strain, neither the 95% CI values of field populations overlapped, nor the resistance ratios (RRs) contained (Tables 1 and 2).

Deltamethrin resistance in O. lybicus field populations. The entire field populations showed some resistance (minor to low levels) against deltamethrin by displaying resistance ratios between 3–10-folds (Table 1). Highest resistance ratio (8.16-fold) was determined in the insects collected from Ba’id with LD50 value of 0.881 ppm and exhibited a low level of resistance against deltamethrin. The insects collected from Al-Ruddah, Al-Ghabbi, Al-Mudhairib, Ghiyazah and Hail Al-Khanabisha also exhibited low levels of resistance against deltamethrin with resistance ratios ranging from 5.08 to 8.16-folds. The O. lybicus strain selected with deltamethrin also displayed a low level of resistance (RR = 7.36-folds) when tested with deltamethrin, with LD50 value of 0.795 ppm. Other populations collected from Miss, Al-Kharma, Sayyah, Al-hajir, Al-Hajar, Al-Waqba, Sint, Falaj Al-Maragha and Al-Wasil exhibited minor levels of resistance against deltamethrin and displayed resistance ratios between 3.22 to 4.70-folds. Comparison of the toxicity values of deltamethrin against field collected populations and LAB-SUS strain demonstrates that no field strain was found susceptible against deltamethrin.

Fenithrothion resistance in O. lybicus field populations. Five out of fifteen O. lybicus field populations unveiled low levels of resistance (RR = 5–10-folds), whereas, eight populations displayed minor levels (RR = 3–5-folds), when the representative toxicity values were compared with those of the LAB-SUS strain against fenithrothion (Table 2). Highest level of resistance (7.22-folds) was determined in the insects collected from Ba’id with LD50 value of 0.065 ppm. The same strain also exhibited a low level of resistance against deltamethrin. Insects collected from Hail Al-Khanabisha, Al-Ruddah, Al-Ghabbi and Ghiyazah also displayed low levels of resistance against fenithrothion with resistance ratios ranging between 5.11 and 5.89-folds. Minor levels of resistance (RR = 3–5-folds) were estimated in the insects collected from Al-Hajir, Miss, Al-Mudhairib, Al-Kharma, Sayyah, Falaj Al-Maragha, Al-Wasil and Al-Waqba. Insects collected from Al-Hajar and Sint were found susceptible by displaying low toxicity scores (RR < 3-folds) and LD50 values of 0.025 and 0.019 ppm, respectively.
A minor level of resistance (RR = 4.33-folds) was also noted in the deltamethrin selected (DEL-SEL) strain with LD₅₀ value of 0.039 ppm and exhibited some evidence of cross resistance.

**Enzyme activities in O. lybicus populations.** The enzyme analyses were performed to detect the activities of CPR, GST and AChE in the field collected, DEL-SEL and LAB-SUS strain of O. lybicus. The analyses revealed significant increased activities of all assayed enzymes [CPR (DF = 16, F = 4.277.74, P = 0.000, R² = 99.95); GST (DF = 16, F = 6.34, P = 0.000, R² = 7.488); AChE (DF = 16, F = 146.79, P = 0.000, R² = 98.57)] in the field collected as well as DEL-SEL strains of O. lybicus when compared with LAB-SUS strain (Table 3).
Cytochrome P-450-Reducase (CPR) activity. Highest CPR activity (293.20 ± 2.32 mU/mg) was observed in the insects collected from Baa’d with an activity ratio of 3.87-folds in comparison with LAB-SUS strain (75.82 ± 3.57 mU/mg), which was followed by the insects collected from Al-Hajir (276.49 ± 3.25 mU/mg) and Al-Ruddah (246.39 ± 2.35 mU/mg) and Al-Ghabbi (238.40 ± 2.81 mU/mg). Lowest enzyme (CPR) activities were observed in insects collected from Al-Waqba (83.95 ± 2.72 mU/mg) and Al-Wasil (113.78 ± 3.65 mU/mg) after the LAB-SUS strain and displayed enzyme activity ratios of 1.11 and 1.50-folds, respectively. Insects collected from Falaj Al-Maragha, Al-Kharma, Ghiyazah, Al-Mudhairib and Hail Al-Khanabisha exhibited a moderate activity of CPR with ratios of 2.86, 2.61, 2.48, 2.34 and 2.05-folds, respectively, when compared with enzyme activity of LAB-SUS *O. lybicus* population. The DEL-SEL strain also displayed an increased level of CPR activity (151.92 ± 3.47 mU/mg) with an activity ratio of 2-folds as compare to the LAB-SUS strain.

Glutathione s-transferase (GST) activity. The insects from DEL-SEL population displayed highest GST activity (82.84 ± 1.76 nmol/min/ml) with a GST activity ratio of 1.93-folds when compared with the enzyme activity of LAB-SUS *O. lybicus* population (42.94 ± 1.33 nmol/min/ml). Amongst the field collected populations, insects collected from Sint and Al-Mudhairib showed highest GST activities (77.53 ± 3.97 nmol/min/ml). Whereas, minimum GST activities were observed in insects collected from Miss, Al-Ruddah, Al-Hajir, Al-Ghabbi, Falaj Al-Maragha, Al-Wasil, Sayyah and Al-Waqba showed moderately higher levels of GST activities with activity ratios of 1.71, 1.71, 1.67, 1.62, 1.61, 1.57 and 1.54-folds, respectively.

Acetyl cholinesterase (AChE) activity. All the field collected populations and the DEL-SEL strain exhibited raised levels of AChE activity as compared to the LAB-SUS strain. Highest levels of AChE activity (210.39 ± 6.65 and 205.44 ± 6.83 U/L) were determined in the insects collected from Baa’d and Hail Al-Khanabisha, respectively, with enzyme activity ratios of 3.10 and 3.03-folds as compare to LAB-SUS *O. lybicus* strain (67.80 ± 1.65 U/L). Minimum AChE activity ratios (1.05, 1.28 and 1.47-folds) were determined in the populations collected from Ghiyazah (55.67 ± 2.29 U/L), Al-Hajir (86.98 ± 1.89 U/L) and Falaj Al-Maragha (99.81 ± 2.44 U/L), respectively. Insects collected from Al-Waqba, Miss and Al-Wasil also exhibited moderately higher AChE activities (198.06 ± 2.03, 190.52 ± 5.05 and 171.65 ± 3.89 U/L, respectively). The DEL-SEL strain also exhibited raised level of AChE activity (139.54 ± 2.59 U/L) with an enzyme activity ratio of 2.06-folds as compare to the LAB-SUS strain of *O. lybicus*.

**Table 3.** Enzyme activities in various field populations of *Ommatissus lybicus*. *Means sharing similar letters in a column do not differ significantly at 5% probability. **Enzyme activity ratios calculated with reference to the LAB-SUS (Reference) strain.

| DB populations     | CPR Activity ± SE (mU/mg)* | Ratio** | GST Activity ± SE (nmol/min/ml)* | Ratio** | AChE Activity ± SE (U/L)* | Ratio** |
|--------------------|-----------------------------|---------|---------------------------------|---------|---------------------------|---------|
| Falaj Al-Maragha   | 216.59 ± 2.84d              | 2.86    | 68.92 ± 1.76abc                | 1.61    | 99.81 ± 2.44gh           | 1.47    |
| Al-Hajir           | 276.49 ± 3.25b              | 3.65    | 71.57 ± 3.04abc                | 1.67    | 86.98 ± 1.89ij          | 1.28    |
| Al-Wasil           | 113.78 ± 3.65 k             | 1.50    | 68.26 ± 4.35abc                | 1.59    | 171.65 ± 3.89c          | 2.53    |
| Al-Kharma          | 197.69 ± 2.31e              | 2.61    | 58.98 ± 2.65bced               | 1.37    | 150.41 ± 3.57d          | 2.22    |
| Miss               | 153.06 ± 1.99h              | 2.02    | 73.56 ± 2.39abc                | 1.71    | 190.52 ± 5.05bc         | 2.81    |
| Ghiyazah           | 187.96 ± 2.72g              | 2.48    | 55.67 ± 2.29cd                 | 1.30    | 71.42 ± 1.77j           | 1.05    |
| Al-Ghabbi          | 238.40 ± 2.81c≤             | 3.14    | 69.58 ± 2.29abc                | 1.62    | 91.96 ± 2.78hi          | 1.36    |
| Al-Ruddah          | 246.39 ± 2.35c              | 3.25    | 73.56 ± 5.94abc                | 1.71    | 123.06 ± 3.97ef         | 1.82    |
| Baal               | 293.20 ± 2.32a              | 3.87    | 62.39 ± 5.26abced              | 1.45    | 210.39 ± 6.65a          | 3.10    |
| Hail Al-Khanabisha | 155.27 ± 4.70h              | 2.05    | 55.00 ± 2.39 ed                | 1.28    | 205.44 ± 6.83ab         | 3.03    |
| Sint               | 140.19 ± 2.48i              | 1.85    | 80.19 ± 1.33ab                 | 1.87    | 110.43 ± 0.86gh         | 1.63    |
| Sayyah             | 167.59 ± 4.03gh             | 2.21    | 67.59 ± 2.30abc                | 1.57    | 145.21 ± 4.17d          | 2.14    |
| Al-Hajar           | 132.18 ± 3.05j              | 1.74    | 58.98 ± 1.33bcd                | 1.37    | 114.42 ± 3.39 fg        | 1.69    |
| Al-Waqba           | 83.95 ± 2.721               | 1.11    | 66.27 ± 2.39abc                | 1.54    | 198.06 ± 2.03ab         | 2.92    |
| Al-Mudhairib       | 177.53 ± 4.35gh             | 2.34    | 77.53 ± 8.97ab                 | 1.81    | 141.50 ± 1.28de         | 2.09    |
| DEL-SEL            | 151.92 ± 3.47hi             | 2.00    | 82.84 ± 1.76a                  | 1.93    | 139.54 ± 2.59de         | 2.06    |
| LAB-SUS            | 75.82 ± 3.57 l              | 42.94 ± 1.33d                             | 67.80 ± 1.65j                             |
| Statistic summary  | DF = 16, F = 4.277.74, P = 0.000, R² = 99.95 | DF = 16, F = 6.34, P = 0.000, R² = 74.88 | DF = 16, F = 146.79, P = 0.000, R² = 98.57 |
Discussion

In Sultanate of Oman, *O. lybicus* is reported to pose serious challenges for devising effective management strategies. Spraying of insecticides is the major pest control option which is carried out through area wide aerial applications using helicopters\(^2\) fitted with ULV spray equipment and through high pressure water pumps for ground and surface applications\(^1\). Emulsifiable concentrate (EC) and ultra-low-volume formulations of organophosphorus and pyrethroid insecticides are more often used for ground and aerial applications, respectively\(^3\). Serious pest infestations in date palm orchards even after heavy aerial and surface spraying confer a challenge for the pest exterminators to devise suitable and environment-friendly pest management strategies\(^4,5\). Over and repeated use of pesticides may cause side effects to the non-target organisms and pose health risks to consumers as well as other organisms by contributing in environmental pollution\(^1,6,7\). Additionally, the injudicious pesticide spraying may result in insecticide resistance development and resurgence of the target and non-target pests. In order to avoid the unnecessary spraying of chemicals, it is necessary to investigate the status of insecticide resistance and the underlying mechanism for its mitigation. The research experiments were carried out to estimate the status of resistance in certain field collected populations of *O. lybicus* and understand the possible role of detoxifying enzymes in insecticide resistance development in the pest.

Results of our experiments demonstrated the indications of field evolved resistance in *O. lybicus* field populations against the tested formulations of insecticides with significantly different resistance levels in contrast with the reference (LAB-SUS) strain. Both insecticides (deltamethrin and fenitrothion) showed highest toxicities to the LAB-SUS reference strain, however, fenitrothion was more toxic amongst the tested insecticides. Susceptibility against deltamethrin was not reported in any field collected *O. lybicus* strain, however, insects collected from Al-Hajar and Sint were found susceptible against fenitrothion in contrast with the toxicity values of the insecticide against LAB-SUS strain. Six out of fifteen dubas bug field populations (Baad, Al-Ruddah, Al-Ghabbi, Al-Mudhairib, Ghizayzah and Hail Al-Khanabisha) exhibited lowest levels of resistance against deltamethrin with resistance ratios ranging from 5.08 to 8.16-folds. The *O. lybicus* strain selected with deltamethrin also displayed a low level of resistance (RR = 7.36-folds) when tested with deltamethrin, whereas remaining nine field populations collected from Miss, Al-Kharma, Sayyah, Al-hajir, Al-Hajar, Al-Waqa, Sint, Falaj Al-Maragha and Al-Wasils exhibited minor levels of resistance against deltamethrin.

Five out of fifteen *O. lybicus* field populations (Baad, Hail Al-Khanabisha, Al-Ruddah, Al-Ghabbi and Ghizayzah) exhibited lowest levels of resistance (RR = 5–10-folds), whereas, eight populations (Al-Hajir, Miss, Al-Mudhairib, Al-Kharma, Sayyah, Falaj Al-Maragha, Al-Wasils and Al-Waqa) displayed minor levels (RR = 3–5-folds), when evaluated with toxicity values of the LAB-SUS strain against fenitrothion. The insects collected from Baad showed a low level of resistance against both tested insecticides. Development of a minor level of resistance (RR = 4.33-folds) was also noted in the deltamethrin selected (DEL-SEL) strain and exhibited some evidence of cross resistance. We have reported previously that the field collected populations did not develop any moderate or higher level of resistance against deltamethrin and fenitrothion, but only one strain collected from Wadi Qari was reported to possess intermediate level resistance against fenitrothion\(^8\). The research presented in this study, however, does not report any field strain to possess intermediate or higher level of resistance against the tested insecticides. A possible reason for the slow development of resistance can be less number of generations in a year and a higher frequency of mutations is crucial for pesticide resistance development\(^9\).

Some field and laboratory studies were performed to appraise and evaluate the toxicity of certain chemicals against *O. lybicus* with the objective to pick the effective one for its management\(^10\). Pyrethroids (deltamethrin, bifenthrin and cypermethrin) were mostly reported as effective insecticides in controlling *O. lybicus* in the field studies\(^11\). However, only one report has surveyed and reported minor and low levels of resistance against deltamethrin and fenitrothion in *O. lybicus*\(^12\). We could not find any report describing the underlying mechanism of resistance development in *O. lybicus*. Elevated and amplified levels of mixed function oxidases in certain pest insects, are reported in literature to aid in resistance development to carbamates, pyrethroids, organochlorines and organophosphates\(^1,3,4,7,31–37\).

In our study, we also performed enzyme activity assays to estimate the activities of detoxifying enzymes viz., CPR, GST and AChE in the field collected, DEL-SEL and LAB-SUS strains of *O. lybicus* to determine their possible role in resistance development. The analyses revealed significant increased activities of all assayed enzymes in the field collected as well as DEL-SEL strains of *O. lybicus* when compared with LAB-SUS strains (DF = 16, P = 0.000). The involvement of CPR in resistance mechanism can be determined because a higher CPR activity was observed in the insects collected from Baad, Al-Hajir, Al-Ruddah and Al-Ghabbi. These populations were found resistant against both insecticides i-e deltamethrin (RR = 8.16-folds) and fenitrothion (RR = 7.22-folds). Lowest enzyme (CPR) activities were observed in insects collected from Al-Waqa and Al-Wasils after the LAB-SUS strain and displayed enzyme activity ratios of 1.11 and 1.50-folds, respectively. These populations exhibited minor levels of resistance against both insecticides. The insects from DEL-SEL population displayed highest GST activity whereas, amongst the field collected populations, insects collected from Sint and Al-Mudhairib showed highest GST activities. In our bioassay experiments, insects collected from these locations also exhibited minor to low levels of resistance. Insects collected from Miss, Al-Ruddah, Al-Hajir, Al-Ghabbi, Al-Wasils, Sayyah and Al-Waqa showed moderately higher levels of GST activities and were found with minor to low levels of resistance in bioassay experiments. Role of GST in resistance to certain insecticides including organophosphates, organochlorines and pyrethroids is also documented and described in many insects including mosquitoes and aphids\(^38,39,41–44\).

All the field collected populations and the DEL-SEL strain exhibited raised levels of AChE activity as compared to the LAB-SUS strain. The AChE activities and role of insensitive and altered AChE in resistance to organophosphates and carbamates have been described in previous studies\(^30–33,40\). Highest levels of AChE activity were determined in the insects collected from Baad and Hail Al-Khanabisha, respectively, with enzyme activity
respectively. deemia tee 21/5, Tallinn, 12,618, Estonia) and BioAssay Systems, 3,191 Corporate Place, Hayward, CA 94,545, Al-Ain, Abu Dhabi, 69,480, United Arab Emirates), Cayman Chemical Company (Cayman Europe OÜ, Aka-

thrin (Decis 2.5EC, Bayer Crop Sciences, Monheim am Rhein, Germany) and fenitrothion (Sumithion 50EC, torate General of Agriculture and Livestock Research, Al-Rumais, Oman provided the insecticide deltamethrin.

The colorimetric enzyme assay kits Sumitomo Chemical, Tokyo, Japan) and acetone (> 90% purity; J.T. Baker, Avantor Performance Materials B.V. Materials and methods

Table 4. List of locations and insect infestation.

ratios of 3.10 and 3.03-folds as compare to LAB-SUS O. lybicus strain. Insects collected from Al-Waqba, Miss and Al-Wasil also exhibited moderately higher AChE activities (198.06 ± 2.03, 190.52 ± 5.05 and 171.65 ± 3.89 U/L, respectively). The raised level of AChE activity (139.54 ± 2.59 U/L) in DEL-SEL strain with an enzyme activity ratio of 2.06-folds describes its involvement in resistance development in field populations of O. lybicus. Conclusively, the study reveals minor to low levels of resistance development in the field populations of O. lybicus. As a consequence of selection pressure under frequent and heavy spray regimes, the pest may develop even higher levels of resistance due to enhanced activities of detoxifying enzymes. Discontinuation of spraying with deltamethrin and fenitrothion and use of alternate chemical pesticides in rotation can help to mitigate the resistance development in O. lybicus. Further studies are also recommended to investigate more efficient and eco-friendly pesticides for inclusion in pest management program in Omani date palm orchards. Less reliance on conventional pesticides and use of biorational insecticides along with some alternate pest management strategies will help in insecticide resistance mitigation and reducing the environmental pollution.

Materials and methods

All the bioassay and enzyme activity assay procedures were performed in the Bioassay Research Laboratory and Entomology Research Laboratory, Plant Protection Research Center, Directorate General of Agriculture and Livestock Research, Al-Rumais, Oman.

Insect collection and rearing. Fifteen field populations of O. lybicus, were collected during 2019 after per-
forming extensive surveys of date palm orchards located in different geographically isolated locations (Table 4, Fig. 1). The insects were collected from the date palm orchards having medium to high pest infestation and were shifted to the mesh cages (80 × 60 × 50 cm) containing potted date palm off-shoots, maintained under the labora-
tory conditions of 27 ± 2 °C, 70 ± 5% RH and 12L:12D photoperiod. A lab strain was used as a reference strain (LAB-SUS) which was collected in 2013 from unsprayed date palms and was maintained without exposing to any insecticide under lab conditions. However, some batches of insects from the LAB-SUS strain were used to select successively by exposing to sub-lethal doses of deltamethrin till seven generations18. The 13th generation of the same DEL-SEL strain was again tested for its resistance status in our current experiments.

Insecticide formulations, solvent and enzyme assay kits. Plant Protection Research Center, Direc-

torate General of Agriculture and Livestock Research, Al-Rumais, Oman provided the insecticide deltameth-

in (Decis 2.5EC, Bayer Crop Sciences, Monheim am Rhein, Germany) and fenitrothion (Sumithion 50EC, Sumitomo Chemical, Tokyo, Japan) and acetone (> 90% purity; J.T. Baker, Avantor Performance Materials B.V. Teugseweg 20-7418AM Deventer, Netherlands) for using in the bioassays. The colorimetric enzyme assay kits (CPR activity, GST activity and AChE activity) were obtained from Abcam (Biomedical Scientific Services LLC, Al-Ain, Abu Dhabi, 69,480, United Arab Emirates), Cayman Chemical Company (Cayman Europe OÜ, Aka-deemia tee 21/5, Tallinn, 12,618, Estonia) and BioAssay Systems, 3,191 Corporate Place, Hayward, CA 94,545, respectively.

Experimental procedure. The residue film bioassay protocols were adapted to observe the toxicity of the selected insecticides (deltamethrin and fenitrothion) against different field collected populations of O. lybi-
cus13,2. A measured volume (1 ml/petri plate) of each dilution (from range of dilutions determined through pre-bioassays and demonstrating 1–99% mortality of the test insect) was applied to the sterilized petri plates and the lid covers. A complete and even application of pesticide surface-residues were obtained by a continu-
ous rotation of petri plates which were then set underneath the fume hood for evaporation. The petri plates in control treatment received acetone (without insecticide). Batches of each strain containing counted number of healthy and homogeneous 3rd instar nymphs were released in the pesticide-treated petri plates. After specified interval (48 h) of insect releases in the treated plates, mortality scores (dead insects) were noted separately and analyzed for comparison. The insects with least movement gestures (appendages) upon physical stimulations with the camel hairbrush were considered alive, and vice versa. The bioassays were performed with all necessary precautions and cares to diminish the experimental error. The data for mortality were corrected by using the abbot’s formula:

\[
\text{Corrected Mortality (\%) } = 100 \times \left(1 - \frac{n (T \text{ after treatment})}{n (Co \text{ after treatment})} \right)
\]

where, \( n \) is number of insects, \( T \) is treated, and \( Co \) is Control.

The enzyme analyses were performed to detect the activities of CPR, GST and AChE in 3rd instar nymphs of field collected, DEL-SEL and LAB-SUS strains of \( O. lybicus \). The protocols provided by the manufacturer were strictly followed while performing the assays. Briefly, for CPR activity, whole body tissues (fresh samples) weighing 5 mg from each strain were homogenized in 500 µL of ice-cold CPR Assay Buffer after washing with Phosphate-buffered saline (PBS). After incubating for five minutes on ice, samples were centrifuged (1,500 × g) at 4 °C. The supernatant from each sample was transferred to pre-chilled microfuge tube and further centrifuged (12,000 × g) at 4 °C for 5 min. The supernatant was again transferred to pre-chilled tubes and stored on ice until CPR activity assay was performed. A standard curve was obtained from standard dilutions (0, 2, 4, 6,
8 and 10 nmol/well) of 1 mM Glucose-6-Phosphate (G6P). Samples of 5 µL G6P standard developer and 5 µL Nicotinamide adenine dinucleotide phosphate (NADPH) were added to the standard wells (containing 90 µL standard 1 mM G6P). After 30 min incubation at room temperature absorbance was measured at an optimal density of 460 nm. The 96-well plate was set up with samples (40 µL sample + 60 µL CPR assay buffer), samples + inhibitor (40 µL sample + 10 µL inhibitor + 50 µL CPR assay buffer), positive control (5 µL Recombinant CPR + 60 µL CPR assay buffer) and background wells (40 µL sample + 2 µL inhibitor + 58 µL CPR assay buffer). To each well, 30 µL of CPR Reaction Mix (5 µL NADPH substrate + 25 µL CPR assay buffer) was added. Finally, 10 µL of 20 mM G6P solution was added to each well except background wells. The absorbance was measured immediately at OD = 460 nm (25 °C) and continued until 30 min in a kinetic mode. The concentration of CPR in the test samples was calculated as

\[
\text{CPR Activity} = \left( \frac{B}{\Delta T \times P} \right) = \text{nmol/min/mg} = \text{mU/mg}
\]

where ‘B’ is the amount of G6P (nmol) consumed (calculated through the standard Curve), ‘\(\Delta T\)’ is the reaction time (minutes) and ‘P’ is the amount of protein sample (mg) added in the reaction well.

The whole-body tissues (3–5 mg) were used and rinsed with PBS (pH 7.4) for performing the GST activity assays. The tissues were then homogenized in 50 ml cold buffer (pH 7.0, containing 2 mM EDTA) and were centrifuged at 10,000 \( \times g \) for 15 min (4 °C). The resultant supernatant was used in GST activity assays. The background wells were set up by adding 170 µL of Assay Buffer and 20 µL Glutathione. The positive control wells contained 150 µL of Assay Buffer, 20 µL of Glutathione and 20 µL of reconstituted GST (control). In sample wells, we added 150 µL of Assay Buffer, 20 µL of Glutathione and 20 µL of samples. The reaction was initiated by adding 10 µL of CDNB (1-chloro-2,4-dinitrobenzene) to each well. The absorbance was measured once every minute at 340 nm at 5 time points. The GST activity was calculated as

\[
\text{GST Activity} = \frac{\Delta A_{340\text{nm}/\text{min},}}{0.00503 \text{ µM}^{-1}} \times \frac{0.02 \text{ ml}}{0.002 \text{ ml}} \times \text{Sample dilution} = \text{nmol/min/ml}
\]

where ‘\(\Delta A_{340}\)’ is the change in absorbance per minute and was determined as

\[
\Delta A_{340\text{nm}/\text{min},} = A_{340\text{nm}(\text{Time2})} - A_{340\text{nm}(\text{Time1})}
\]

where ‘\(A_{340\text{nm}(\text{Time2})}\)’ and ‘\(A_{340\text{nm}(\text{Time1})}\)’ are the OD412nm values of samples at 10 min and 2 min, respectively. ‘\(OD_{\text{CAL}}\)’ and ‘\(OD_{\text{H2O}}\)’ are the OD412nm values of Calibrator and water at 10 min. The number ‘200’ is the equivalent activity of the calibrator under assay conditions.

Data analyses. The toxic dose values (LD90) of deltamethrin for test populations, resistance ratios over susceptible (Reference) strain and their confidence interval values were estimated by performing the Probit Analysis\(^{54}\) of the recorded mortalities in each experiment using Polo Plus software version 1.0 (LeOra software LLC). Treatments with non-overlapping confidence intervals were considered significantly different for their toxicities (LD90). Higher slope numbers (> 0.90) described lower dissimilarity in physical appearances of the treated insects from each field strain\(^{55}\). The resistance ratios (RRs) were calculated through the division of median lethal dose of the respective (field/selected) strain and median lethal dose the reference strain. Categories for resistance were determined by following the scale described by Jin and his co-workers\(^{56}\) i.e susceptible (RR < 3 folds), minor resistance (3 ≤ RR ≤ 5 folds), low resistance (5 ≤ RR ≤ 10 folds), intermediate resistance (10 ≤ RR ≤ 40 folds), high resistance (40 ≤ RR ≤ 160 folds) and extremely high resistance (RR > 160 folds). The values of RRs were measured significantly different only when 95% CI (confidence interval) did not include 1 \(^{54}\). The results obtained in enzyme activity studies were analyzed for variance and the means were compared by Tukey’s HSD test through Minitab 18 software.

Ethical statement
Any author did not perform any experiment with humans as participants. All the standard protocols for performing the bioassay and enzyme activity experiments with the insect O. lybicus belonging to phylum Arthropoda, were performed after approval from the Plant Protection Research Center, Ministry of Agriculture and Fisheries, Oman.
References
1. Nauen, R. & Elbert, A. European monitoring of resistance to insecticides in Myzus persicae and Aphis gossypii (Hemiptera: Aphididae) with special reference to imidacloprid. Bull. Entomol. Res. 93, 47–54 (2003).
2. Khan, H. A. A. Characterization of permethrin resistance in a Musca domestica strain: resistance development, cross-resistance potential and realized heritability. Pest Manag. Sci. 75, 2969–2974 (2019).
3. Khan, H. A. A. et al. Risk assessment, cross-resistance potential, and biochemical mechanism of resistance to emamectin benzoate in a field strain of house fly (Musca domestica Linnaeus). Chemosphere 151, 133–137 (2016).
4. Chauzat, M. & Faucon, J. Pesticide residues in beeswax samples collected from honey bee colonies (Apis mellifera L.) in France. Pest Manag. Sci. 63, 1100–1106 (2007).
5. Barganska, Z., Slebioda, M. & Namiesnik, J. Determination of pesticide residues in honeybees using modified quick, easy, cheap, effective, rugged and safe (QUEChERS) sample work-up approach and liquid chromatography-tandem mass spectrometry. Molecules 19, 2911–2924 (2014).
6. Resistance risk assessment and bio-chemical mechanism. Khan, H. A. A. & Akram, W. Cyromazine resistance in a field strain of house flies, Musca domestica L. Chemosphere 167, 308–313 (2017).
7. Shahani, F., Kumar, L. & Al Shidi, R. H. S. Impacts of climate change on infestations of Dubas bug (Ommatissus lybicus Bergevin) on date palms in Oman. PeerJ 6, e5545. https://doi.org/10.7717/peerj.5545 (2018).
8. Al Shidi, R. H., Kumar, L. & Al-Khatri, S. A. H. Detecting Dubas bug infestations using high resolution multispectral satellite data in Oman. Comput. Electron. Agric. 157, 1–11 (2019).
9. Al-Kindi, K. M., Khan, W. A. & Welch, M. Impact of environmental variables on Dubas bug infestation rate: a case study from the Sultanate of Oman. PLoS ONE 12, 0178109 (2017).
10. Mamoon, A., Wright, D. J. & Dobson, H. Assessing the optimum droplet size for controlling Dubas bug on Date Palm in the Sultanate of Oman when applying an insecticide spray from an aircraft. Outlooks Pest Manag. 27, 111–115 (2016).
11. Khalaf, M. Z. & Khudhair, M. W. Spatial distribution of dubas bug, Ommatissus lybicus (Homoptera: Tropiduchidae) in date palm fronts in the coastal areas of Hadramout governorate, Republic of Yemen. Pak. J. Zool. 39, 1–13 (2015).
12. Al-Shidi, R., Kumar, L. & Al-Khatri, S. A. Humid-thermal index for a new management approach of Ommatissus lybicus. Pest Manag. Sci. 75, 3060–3069 (2019).
13. Al Shidi, R. H., Kumar, L., Al-Khatri, S. A. H., Alaufi, M. S. & Alabahi, M. M. Does solar radiation affect the distribution of dubas bug (Ommatissus lybicus de Bergevin) infestation? Agriculture 8, 4–8 (2018).
14. Al-Kindi, K. M., Khan, W. A. & Welch, M. Impacts of human-related practices on Ommatissus lybicus infestations of date palm in Oman. PLoS ONE 12, 1–17 (2017).
15. Shah, A. et al. Biology of Dubas Bug, Ommatissus lybicus (Homoptera: Tropiduchidae), a pest on date palm during spring and summer seasons in Jazan, Saudi Arabia. J. Arid Land Environ. 5, 160–167 (2011).
16. Ali, A. & Hama, N. Integrated management for major palm disease pests in Iraq. Emamet Al Elem疼痛, 133–137 (2016).
17. Karimi, S., Izadi, H., Askari Seyedhosei, M., Bagheri, A. & Khodaygan, P. Variation in bacterial endosymbionts associated with the date palm hopper, Ommatissus lybicus populations. Bull. Entomol. Res. 108, 271–281 (2018).
18. Khan, R. R. et al. Susceptibility survey of Ommatissus lybicus (de Bergevin) populations against deltamethrin and fenithrothion in Oman. Sci. Rep. 9, 11690 (2019).
19. Bagheri, A., Fathipour, Y., Askari Seyedhosei, M. & Zeinalabedini, M. Ommatissus lybicus (Homoptera: Tropiduchidae), an economically important pest of date palm (Araceae) with highly divergent populations. Can. Entomol. 150, 378–392 (2018).
20. Abbaszadeh, G. Efficacy of different insecticides against dubas bug, Ommatissus lybicus (Debgerberg Aschae & Wilson). Ann. Plant Prot. Sci. 22, 240–243 (2014).
21. Al-jamali, N. A. & Kareem, T. A. Evaluation the efficiency of spinetoram 125g against dubas bug on the date palm Ommatissus binotatus (Homoptera : Tropiduchidae). In Proceedings of the 7th international date palm conference 467–470 (2014).
22. Mahmoudi, M., Sahragard, A., Pehziman, H. & Ghadamyari, M. Efficacy of biotic insecticides against dubas bug, Ommatissus lybicus (Homoptera: Hemi: Tropiduchidae) in a date palm orchard and evaluation of kaolin and mineral oil in the laboratory. J. Entomol. Soci. Iran 33, 1–10 (2014).
23. Salim, H. A., Zewain, Q. K., Hassan, K. A. & Salman, M. M. Effectiveness of Telstar and Decis insecticides against dubas bug, Ommatissus binotatus f. sp. lybicus (Homoptera: Tropiduchidae) at Diyaly Governorate, Iraq. J. Genet. Environ. Resour. Conserv. 5, 24–27 (2017).
24. Rashomaila, S. M., Al-Jboory, I. J. & Madi, A. O. Field Efficacy of bio-rational pesticide fytomax N against dubas bug Ommatissus lybicus de Berg (Homoptera : Tropiduchidae) in autumn and spring generation. In Proceedings of the 7th international date palm conference 387–392 (2014).
25. Hamad, B. & Al-Raway, M. Toxicity of Deltamethrin and Basudin to Chrysoperla mutata MacL. and Dubas nymphs Ommatissus lybicus DeBerg. Arab J. Plant Prot. 27, 210–213 (2009).
26. Al-Rawahi, F. G., Al-antary, T. M. & Saddar, M. T. Toxicity of three chemicals and neem insecticides against spring populations of dubas bug, Ommatissus lybicus de Bergevin (Tropiduchidae: Homoptera) in Oman. Presentus Environ. Bull. 27, 8594–8599 (2018).
27. Al-Rawahi, F. G., Al-antary, T. M. & Saddar, M. T. Effect of three chemicals and neem insecticide against autumn populations of dubas bug, Ommatissus lybicus de Bergevin (Tropiduchidae: Homoptera) in Oman. Presentus Environ. Bull. 27, 8590–8594 (2018).
28. Ba-Angood, S. Biology and chemical control of the old world bug (Dubas bug) Ommatissus lybicus Deberg on date palm trees in the coastal areas of Hadramout governorate, Republic of Yemen. Arab J. Plant Prot. 27, 1–9 (2009).
29. Markussen, M. D. K. & Kristensen, M. Spinosad resistance in female Musca domestica L. from a field-derived population. Pest Manag. Sci. 68, 75–82 (2012).
30. Low, V. L. et al. Enzymatic characterization of insecticide resistance mechanisms in field populations of Malaysian Culex quinquefasciatus Say (Diptera: Culicidae). PLoS ONE 8, 1–8 (2013).
31. Fournier, D. & Muteru, A. Modification of acetylcholinesterase as a mechanism of resistance to insecticides. Comp. Biochem. Physiol. Part C Pharmacol. 108, 19–31 (1994).
32. Zayed, A. B. et al. Use of biosassay and microscopic assay to detect and measure insecticide resistance in field populations of Culex pipiens from filariasis endemic areas of Egypt. J. Am. Mosq. Control Assoc. 22, 473–482 (2006).
33. Villatte, F., Ziliani, P., Marcel, V., Menozzi, P. & Fournier, D. A high number of mutations in insect acetyl cholinesterase may provide insecticide resistance. *Pestic. Biochem. Physiol.* **67**, 95–102 (2000).

34. Devonshire, A. L. & Moores, G. D. A carboxylesterase with broad substrate specificity causes organophosphorus, carbamate and pyrethroid resistance in peach-potato aphids (Myzus persicae). *Pestic. Biochem. Physiol.* **18**, 235–246 (1982).

35. Che-Mendoza, A., Penilla, R. P. & Rodriguez, D. A. Enzymes mediating resistance to lambda-cyhalothrin in *Triatoma infestans*. *Pestic. Biochem. Physiol.* **110**, 36–43 (2014).

36. Kang, C. Y., Wu, G. & Miyata, T. Synergism of enzyme inhibitors and mechanisms of insecticide resistance in *Bemisia tabaci* (Hom., Aleyrodidae). *J. Appl. Entomol.* **130**, 377–385 (2006).

37. Robertson, J. L., Jones, M. M., Olguin, E. & Brad, A. Bioassay with arthropods. *J. Econ. Entomol.* **94**, 837–846 (2016).

38. Abbreviations

38. Abbott, W. S. A Method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **18**, 256–267 (1925).

39. Robertson, J. L., Jones, M. M., Olguin, E. & Brad, A. Bioassay with arthropods (CRC Press, Broken Sound Parkway, 2017).

40. Achaleke, J., Martin, T., Ghogomu, R. T., Vaissayre, M. & Brévault, T. Esterase-mediated resistance to pyrethroids in field populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from Central Africa. *Pest Manag. Sci.* **65**, 1147–1154 (2009).

41. Kang, C. Y., Wu, G. & Miyata, T. Synergism of enzyme inhibitors and mechanisms of insecticide resistance in *Bemisia tabaci* (Hom., Aleyrodidae). *J. Appl. Entomol.* **130**, 377–385 (2006).

42. Ni, X. & Quisenberry, S. S. Possible roles of esterase, glutathione S-transferase, and superoxide dismutase activities in understanding aphid-cereal interactions. *Entomol. Exp. Appl.* **108**, 187–195 (2003).

43. Aiello, L., Savantini, P., Cammarota, F. & Belli, G. Effect of temperature on the development and survival of *Bactrocera oleae* (Diptera: Tephritidae). *Bull. Entomol. Res.* **91**, 265–272 (2001).

44. Ahmad, S. & Ahmad, M. Note: Toxicity of some insecticides on *Bracon hebetor* under laboratory conditions. *J. Econ. Entomol.* **91**, 256–267 (2001).

45. Hawkins, N. J., Bass, C., Dixon, A. & Neve, P. The evolutionary origins of pesticide resistance. *Biol. Rev.* **94**, 135–155 (2019).

46. MAF. Efficacy of two insecticides against dubas bug *Ommatissus lybicus* De Bergevin during spring generation. *Directorate General of Agriculture and Livestock Research, Annual Report 2008* (2008).

47. MAF. Efficacy of some insecticides against dubas bug *Ommatissus lybicus* De Bergevin during spring generation. *Directorate General of Agriculture and Livestock Research, Annual Report 2009* (2009).

48. El-Shafei, H. A., El-Sayed, M. & El-Tabbakh, M. Effects of different insecticides on the development and survival of *Bactrocera oleae* (Diptera: Tephritidae). *Bull. Entomol. Res.* **91**, 265–272 (2001).

49. Hawkins, N. J., Bass, C., Dixon, A. & Neve, P. The evolutionary origins of pesticide resistance. *Biol. Rev.* **94**, 135–155 (2019).

50. Fournier, D. Mutations of acetylcholinesterase which confer insecticide resistance in insect populations. *Chem. Biol. Interact.* **157–158**, 257–261 (2005).

51. Dasgupta, S. & Maiti, S. The effect of different insecticides on the development and survival of *Bactrocera oleae* (Diptera: Tephritidae). *Bull. Entomol. Res.* **91**, 265–272 (2001).

52. Gonzalez-Zamora, J. E., Castillo, M. L. & Avila, C. Side effects of different pesticides used in citrus on the adult stage of the parasitoid *Aphyis melinus* DeBach (Hymenoptera: Aphiaphelimidae) and its progeny. *Spanish J. Agric. Res.* **11**, 494–504 (2013).

53. Abbott, W. S. A Method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **18**, 256–267 (1925).

54. Robertson, J. L., Jones, M. M., Olguin, E. & Brad, A. Bioassay with arthropods (CRC Press, Broken Sound Parkway, 2017).

55. Ahmad, S. & Ahmad, M. Note: Toxicity of some insecticides on *Bracon hebetor* under laboratory conditions. *Phytoparasitica* **34**, 401–404 (2006).

56. Jin, T., Lin, Y. J., Jin, Q. A., Wen, H. B. & Peng, Z. Q. Population susceptibility to insecticides and the development of resistance in *bactrocera cucurbitae* (Diptera: Tephritidae). *J. Econ. Entomol.* **109**, 837–846 (2016).

**Acknowledgments**

Authors are thankful to The Research Council (TRC), Sultanate of Oman for supporting the research studies and providing financial grant under contract number TRC/SRG/DB/13/003.

**Author contributions**

R.R.K. conceived the idea and together with T.H.A.A and I.S.S.A designed the experiments. T.H.A.A conducted the field surveys and collected the field populations of dubas bug. I.S.S.A and F.A performed the enzyme assays. R.R.K and S.A.H.A performed the statistical analysis of data and wrote the first draft of manuscript. R.R.K prepared the map for insect collection sites. All authors reviewed the drafts of paper and approve the final draft.

**Competing interests**

The authors declare that they have no competing interests.

**Additional information**

**Correspondence** and requests for materials should be addressed to R.R.K.

**Reprints and permissions information** is available at www.nature.com/reprints.

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020