IPS Journal of Applied Microbiology and Biotechnology
ISSN: 3115-5685
IPS J Appl Microbiol Biotech, 6(2): 440-447 (2026)
DOI: https://doi.org/10.54117/ijamb.v6i2.159

An Ultraviolet Light-Assisted Method for the Retrieval of Fluorescent Implanted Monofilaments in Insect Larvae for Encapsulation Assays Using *Zophobas morio* as a Model

Bridget Ugochi Anyanwueze[1*], Maria Ada Ameh[2], Paul Uchechukwu Ebo[3]

[1]Department of Biology, Southeast Missouri State University, Cape Girardeau, MO, USA.
[2]Department of Microbiology, Tansian University, Umunya, Anambra State, Nigeria.

*Corresponding author *e-mail address: bridgetugochi4@gmail.com*

Abstract

Encapsulation assays are widely used in eco immunology to assess insect immune responses against foreign bodies and simulated parasitic challenges. However, locating implanted monofilaments during recovery can be difficult, time consuming, and highly dependent on the experience and mastery of the observer, particularly in conditions where surrounding tissues make the filament difficult to see for retrieval. This study presents a simple and cost-effective ultraviolet assisted method for improving the localization of implanted ultraviolet reactive monofilaments in insect larvae using fluorescent fishing lines. Using *Zophobas morio* as a model organism, detection time was compared under conventional lighting using the microscope and UV illumination across conditions with different surrounding tissues. Detection time served as a measure of retrieval efficiency. Ultraviolet illumination substantially reduced the time required to locate and retrieve implanted monofilaments, with the greatest improvements observed in cellulose-treated larvae, where surrounding tissues made filament detection more difficult and detection time decreased by approximately 57–86%. The differences between the two detection methods were smaller and less consistent under wheat bran conditions. Successful monofilament recovery was achieved across all treatments, demonstrating that the ultraviolet assisted approach improves efficiency, with the new method providing a quick, accessible, and reproducible approach. Because of its simplicity, this method can be used in host–pathogen studies and other research involving implanting objects to mimic foreign bodies.

Keywords: Encapsulation assay, ultraviolet light (UV), monofilament retrieval, *Zophobas morio*, insect immunity, detection efficiency, fluorescent fishing line, improved method.

Article History
Received: 08 Mar 2026
Accepted: 23 Apr 2026
Revised: 15 May 2026
Published: 19 May 2026

Scan QR code to view•

How to cite this paper: Anyanwueze, B. U., Ameh, M. A., & Ebo, P. U. (2026). An Ultraviolet Light-Assisted Method for the Retrieval of Fluorescent Implanted Monofilaments in Insect Larvae for Encapsulation Assays Using Zophobas morio as a Model. *IPS Journal of Applied Microbiology and Biotechnology*, *6*(2), 440–447. https://doi.org/10.54117/ijamb.v6i2.159

1. Introduction

Organisms constantly face tradeoffs in allocating limited resources among growth, reproduction, body maintenance, and immune defense. These tradeoffs are important because immune responses are energetically costly and can be influenced by extrinsic conditions such as diet, temperature, and pathogen exposure. Considering how fast the field of eco immunology is advancing, it examines the ecological and evolutionary factors and how they influence variation in immune function among organisms (Downs and Stewart, 2014; Schoenle *et al.*, 2018; Ohmer *et al.*, 2021). As a result, ecologists in this field increasingly rely on mechanism of the immune system to better understand the extent to which these extrinsic conditions affect immune responses across different species. This mutual adaptive interaction has forced hosts to develop complex defense mechanisms, with the immune system serving as one of the primary protective systems (Freitak *et al.*, 2003). Despite the importance of immune defense, organisms usually have limited resources, and energy devoted to immune responses may reduce the resources available for other physiological functions including growth, survival, and reproduction (Williams, 1966; Strand, 2008; Daniel *et al*., 2012; Krams *et al*., 2015).

Evidence indicates that immune responses are connected to fundamental life history tradeoffs involving survival, growth, and reproduction (Schmid- Hempel, 2003; Freitak *et al.*, 2003; Daniel *et al.*, 2012), with this relationship revealing the energetic demands associated with immune activation and the need for organisms to balance immunity with life trade history

(Strand, 2008; Rapkin *et al*., 2018). Although invertebrates' immune response has been extensively investigated for decades, research on immunity in invertebrates has received comparatively less attention (Fellowes and Godfray, 2000; Ayres & Schneider, 2009). Increasing interest in insect immune systems has expanded understanding of how invertebrates respond to pathogens, parasites, and other foreign materials under varying environmental conditions (Demas *et al*. 2011). The relationship between immune function and reproduction may not be at same rate among species due to their various encounter with pressure from their environment. (Ryder and Siva-Jothy, 2001; Rantala *et al*., 2003; Kiss *et al.*, 2020).

Studies on insect immunity have shown that inbreeding does not always reduce immune performance or disease resistance, as observed in the termite *Zootermopsis angusticollis*, where inbreeding had no effect on encapsulation response or resistance to bacterial and fungal infections, and similarly in the flour beetle *Tribolium castaneum*, where inbred lines displayed resistance to parasitic nematodes comparable to that of stock populations (Calleri *et al*., 2006; Stevens *et al*., 1997), there has also been report in the sand cricket *Gryllus firmus*, where individuals from inbred lines were smaller and required longer developmental periods than individuals from crossbred lines, yet showed no significant differences in immune function (Rantala and Roff, 2006).

Encapsulation is an important cellular immune response in insects used to defend against foreign bodies and invading foreign object that cannot be eliminated through phagocytosis alone (Brennan & Anderson, 2004; Ojala *et al*., 2005; Moreno-Garcia *et al*., 2013; Krams *et al*., 2013), during this process, multiple hemocytes migrate to the surface of the implanted or invading object, attach to it and gradually form cellular layers around the target (Qiu & Govind, 1998; Zettervall *et al.*, 2004). This response is followed by melanization and the deposition of immune-related compounds that help isolate and neutralize the foreign material (Dubovskiy *et al*., 2016; Gupta, 2019), this assays is commonly used to evaluate insect immune defenses against implanted foreign materials and simulated parasitic challenges(Pham *et al.*, 2007). In addition, antibacterial responses, including the production of antimicrobial peptides (AMPs), represent another important component of insect immunity, playing a critical role in protection against bacterial pathogens and parasitic infections (Pham *et al.*, 2007; Hultmark, 1993; Gupta, 2019). These processes are involved in melanin production, which plays an important role in insect cuticle pigmentation and in the immune defense mechanism used to surround and isolate foreign materials within the hemolymph (Götz, 1986; Gupta, 2019). The host preference of *A. tabida* differs across geographic regions depending. Differences in encapsulation ability among insect hosts can influence parasite success and shape host–parasite interactions within natural populations (Lowenberger *et al*., 1999; Kraaijeveld and Godfray, 1999).

This variation in host selection depicts the importance of encapsulation as a defensive immune mechanism that can influence host susceptibility, parasite success, and host–parasite interactions amongst population in the ecosystem (Johnsen and Zuk, 1999; Joyce *et al.*, 2004; Schmid-Hempel, 2005; Graham *et al*., 2011).

The retrieval of implanted monofilaments involves dissection and several manipulations on the internal body cavity to find these implants. This is a big challenge that is encountered in both large and small invertebrates because these implants tend to shift positions within the body cavity while the invertebrate makes movement as well as when immune response begins to occur the filament becomes surrounded by haemocoel and immune deposits covering the implant from being visible. Therefore, improving the technique to this retrieval can make carrying out encapsulation assays efficiently used in eco-immunology studies. Since detecting monofilaments or objects used for immune assay takes so much time, it is advantageous to develop a more reliable method to retrieve monofilament to improve the efficiency of this assay. Such improvements are valuable for researchers using implanted objects to mimic foreign bodies in studies investigating invertebrate immunity, host–pathogen interactions, and physiological stress responses. In this paper, we present an enhanced technique to easily locate monofilaments used in encapsulation assays, with the blue light beam from the 395-405 nm ultraviolet light, with this method aiming to streamline and accelerate monofilament retrieval. To determine its effectiveness, we compared the ultraviolet light-based retrieval method with the conventional retrieval method (microscope light).

2. Methods

2.1 Experimental Setup

A total of forty larvae were purchased from a commercial supplier and randomly assigned to two diet treatments. Twenty larvae were fed wheat bran (calorie-enriched diet) and twenty were fed cellulose (calorie-restricted diet). A handheld ultraviolet torch used during monofilament retrieval is shown in Fig. 1. Larvae were implanted 2mm monofilament (Fig. 2), into their third segment from the posterior end using a sterile injection pen and left in their respective diet for 24hours to initiate immune response. At the end of the 24 hours, larvae were frozen at −80°C for 24 hours, allowed to thaw and later dissected to retrieve monofilaments.

2.2 Comparison of conventional and ultraviolet localization methods

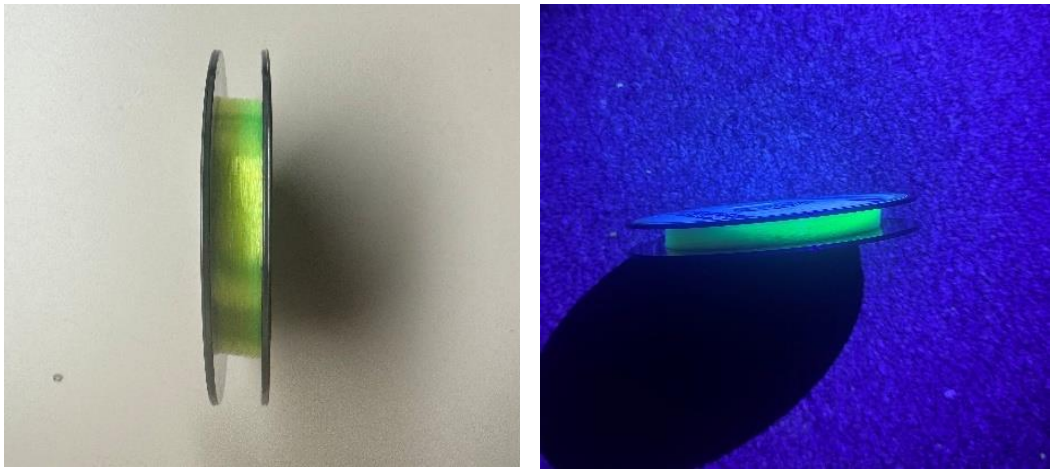
To evaluate the effectiveness of ultraviolet assisted retrieval, the time required to locate implanted monofilaments was compared with conventional light and ultraviolet light. Under conventional lighting, larvae were examined using microscope light, whereas under ultraviolet light, a handheld ultraviolet torch was used. Two observers conducted this trial. The first observer had one year and one month of experience with encapsulation assays and monofilament retrieval using conventional lighting, whereas the second observer had only two weeks of experience with encapsulation assays before

using the ultraviolet light-assisted retrieval method. This essence for using two observers was to evaluate whether the ultraviolet-assisted method could improve retrieval efficiency across observers with different levels of experience. For each larva, the time required to locate the implanted monofilament was recorded. Retrieval times were compared across both methods and diet treatments to determine whether ultraviolet illumination could improve detection speed of the monofilaments.

2.3 Retrieval Procedure using ultraviolet

During the monofilament retrieval, the handheld ultraviolet torch (Fig. 1a), with the beam of light (Fig. 1b), pointed towards the body of each larva with implants from approximately 5–10 cm. Observations were performed under a microscope with screen display to allow magnification.

Figure 1: (a) Ultra Fire (395-405nm) handheld torch **(b)** Ultra Fire (395-405nm) handheld torch showing the emitted light beam used to enhance visibility of implanted monofilaments during localization.



a. **b.**

Figure 2: (a) Monofilament fishing line (Reactive tackle high performance fishing line) before exposure to ultraviolet light. **(b)** Monofilament fishing line after exposure to ultraviolet light, showing fluorescence due to embedded dye.

2.4 Comparison of Filament Visibility under Different Lighting Conditions

Filament visibility under both lighting conditions was documented by capturing side-by-side images of the same implanted larva first under conventional microscope light and then under ultraviolet illumination, to qualitatively illustrate the difference in contrast and localization clarity between the two methods (Fig. 3).

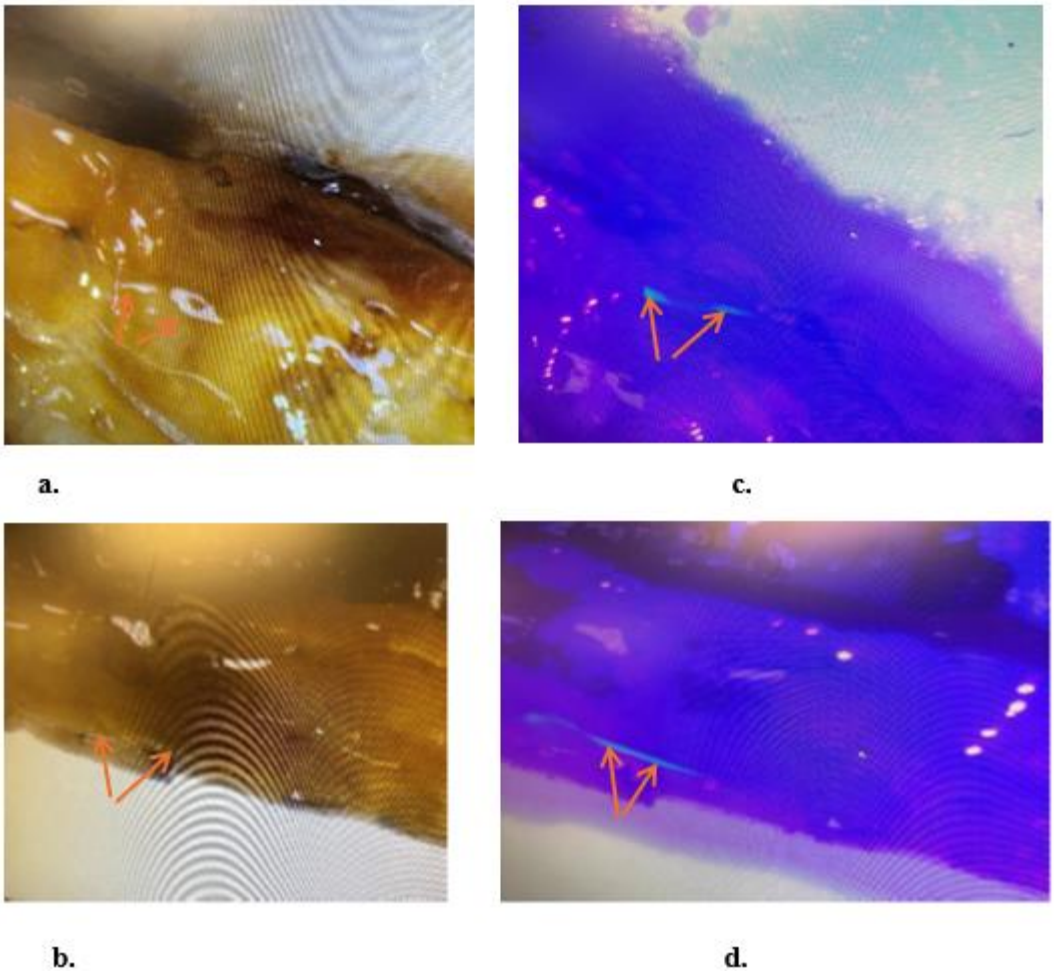


Figure 3: Side-by-side comparison of the filament localization methods. **(a & b)** Implanted monofilament is not clearly visible under microscope light. **(c & d)** Implanted monofilament visible in cyan blue with the assistance of ultraviolet light.

2.5 Statistical Analysis

Detection time was used to evaluate the efficiency of monofilament retrieval under UV illumination and conventional light. All analyses were conducted in All analyses were conducted using R and RStudio software. Analyses were performed separately for each observer and diet condition, with each comparison based on five observations per detection method (UV and conventional light; n = 5 per group). Because of the small sample size and variability in detection time, differences between observation methods were primarily assessed using the Wilcoxon rank-sum test (Mann–Whitney U test). In cases where data met approximate normality assumptions, Welch's t-test was additionally applied. Statistical significance was set at $p < 0.05$. Detection times are presented as medians with ranges, and box plots were used to visualize the distribution of values. In the box plots, higher positions indicate longer detection times (slower location), whereas lower positions indicate shorter detection times (faster location). The spread of each box reflects variability in detection time, broader boxes indicate greater variation and lower consistency among observations, while narrower boxes indicate more consistent detection times across trials.

3. Results

During retrieval using the conventional procedure, the monofilament was not distinguishable from the surrounding tissues (Fig. 3a &b), while under ultraviolet illumination the implanted monofilament appeared more distinct against surrounding tissues in cyan blue (Fig 3 c & d), using the microscope with display on screen to aid magnification.

3.1 Method Effects Figures description

The detection times under conventional lighting are higher and more spread out among the larvae, indicating greater variation, while detection times under ultraviolet light are lower and more closely grouped, indicating faster and more consistent detection and retrieval (Fig. 4a), detection times under conventional lighting vary widely among the larvae, with both low and high values, whereas detection times under ultra violet light are generally lower and more consistent (Fig. 4b). Under conventional lighting the times are widespread among the larvae, indicating high variability, while detection times under ultraviolet light still vary but are more closely grouped within a moderate range (Fig. 4c). The conventional lighting exhibits

some variation among the larvae, whereas detection times under ultraviolet light vary widely, with both low and high values, indicating less consistency (Fig. 4d).

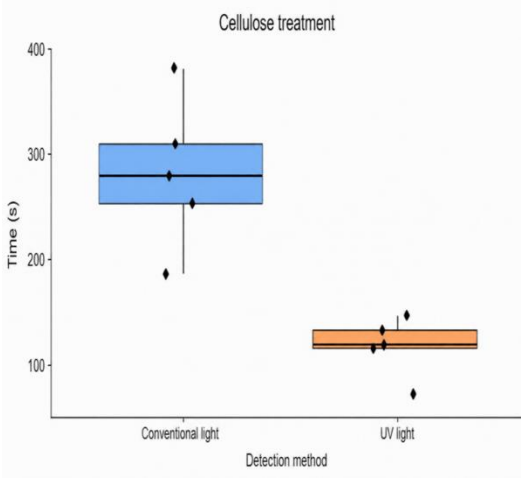
The time for detection varied across diet conditions or surrounding tissue conditions, with the most consistent effect of ultraviolet illumination observed under cellulose conditions. For the first observer, (Fig. 4a) cellulose treatment, median detection time decreased from 279 seconds under conventional lighting to 121 seconds under ultraviolet illumination, representing a highly significant improvement and difference (W = 25, p-value = 0.007937). A similar pattern was observed in second observer, cellulose treatment Fig. 4d, where detection time decreased from 266 seconds to 38 seconds under ultraviolet illumination, also showing a highly significant difference (W = 25, p-value = 0.007937). On the other hand, under wheat bran conditions, the advantage of ultraviolet illumination was reduced. In Fig. 4b (first observer) wheat bran treatment, detection time decreased from 149 seconds under conventional lighting to 106 seconds under ultraviolet illumination; however, this difference was not statistically significant (W = 18, p-value = 0.3095). Similarly, in (Fig. 4c) wheat bran (second observer), detection times were comparable between methods, with median values of 93 seconds under conventional lighting and 115 seconds under ultraviolet illumination, showing no significant difference (W = 13, p =0.8405).

It is important to note that larvae from each wheat bran group died. Two larvae died from the ultraviolet detection group in (Fig. 4c), and one larva died from ultraviolet detection group for (Fig. 4b), and their gut tissues became very dark, making it difficult to detect monofilament. This likely contributed to the increased variability and reduced effectiveness of ultraviolet illumination observed in wheat bran conditions compared to conventional light, aside from these cases monofilaments were generally easier to detect under ultraviolet light. These results are an indication that ultraviolet assisted retrieval is effective and faster for detecting implanted monofilaments, under conditions where the filament was hard to see.

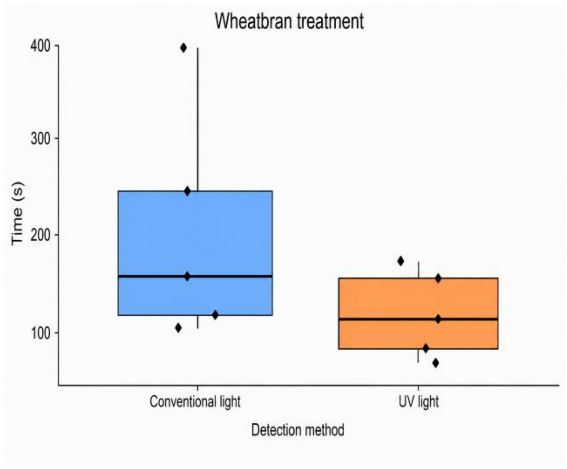
3.2 Diet Treatment Effects Figures description

The (Fig. 5a) depicts that detection times under ultraviolet light are moderate and consistent for both cellulose and wheat bran, although wheat bran shows slightly more variation, while (Fig. 5b) shows that under conventional lighting, detection times in cellulose are higher and more grouped, while detection times in wheat bran had large variance between larvae in that group and detection time generally lower than the cellulose group. Under ultraviolet illumination (Fig. 5a), detection time did not differ significantly between cellulose and wheat bran treatments (p = 0.436). The median detection time was 96.5 s in cellulose and 110.5 s in wheat bran, lack of statistical significance may have been influenced by three dead larvae, which increased the time required for monofilament retrieval in some samples.

However, under conventional lighting (Fig. 5b), detection time was statistically significantly longer in cellulose-treated larvae than in wheat bran-treated larvae (p = 0.026). Median detection times were 269.5s for cellulose and 129.5 s for wheat bran, indicating that filament detection and retrieval was more difficult under cellulose conditions when using conventional light.

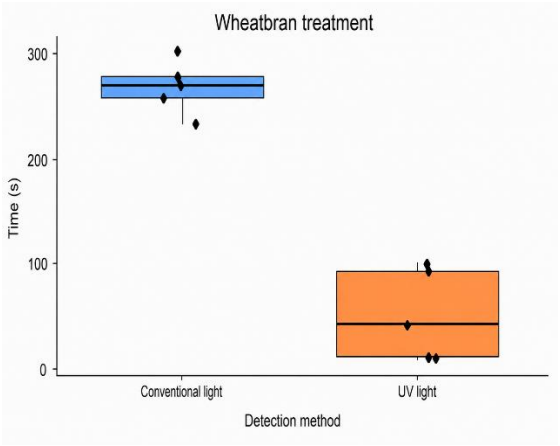


4a

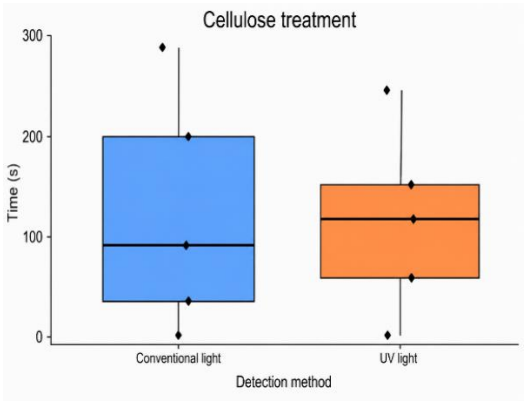


4b.

Figure 4: Detection time (s) for monofilament localization under conventional lighting and ultraviolet (UV) illumination across cellulose (a, d) and wheat bran (b, c) diet treatments. Lines within boxes indicate medians, boxes represent the 25th and 75th percentiles, and whiskers extend to 1.5× the interquartile range.



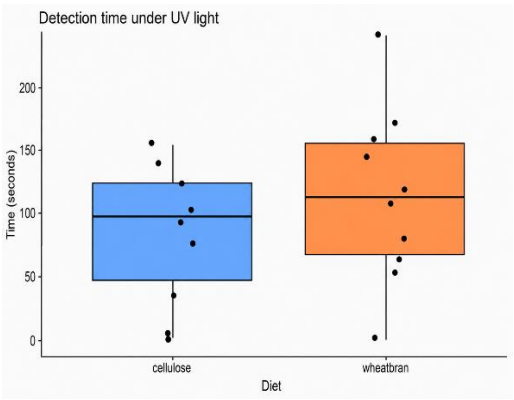
4c.



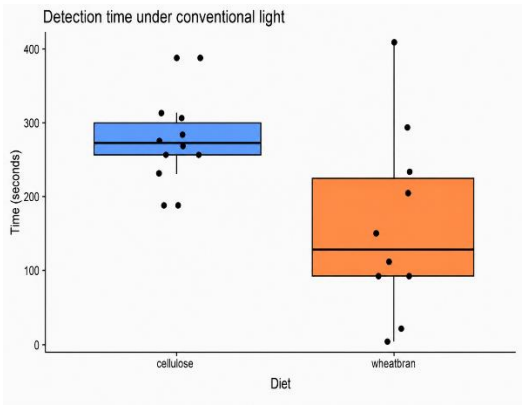
4d.

Figure 4: (Cont'd).

.



5a.



5b.

Figure 5: (a) Detection time (s) for monofilament localization under UV illumination and **(b)** Conventional light across cellulose and wheat bran treatments. Lines inside boxes indicate medians, boxes indicate the 25th and 75th percentiles, bars indicate the interquartile range of 1.5, and points represent individual measurements.

4. Discussion

This study introduces a unique approach for locating implanted monofilaments during immune response assays, and studies that require implants as a marker demonstrating significant improvements in detection efficiency. With the use of ultraviolet illumination, observers achieved a remarkable reduction in the time required to locate implanted filaments compared to conventional observation methods. In cellulose-fed larvae, where background visibility is limited and visual contrast is low, detection times decreased by approximately 57–86%. This pronounced improvement was most evident in trials conducted in low-contrast environments, emphasizing the effectiveness of ultraviolet assisted localization under these specific conditions. However, filament recovery can be a time-intensive step and may be subject to observer-dependent variation, particularly when it is harder to clearly see the filament against the surrounding tissue.

This conventional method requires a special type of mastery to locate monofilament, but our method makes it easier for those who has little or no experience, since all is required to do is to dissect the study organism around the region the monofilament wash inserted and flash the ultraviolet light, then the filament glows immediately and the ultraviolet light hits it, because the second observer who does not have sufficient experience was still able to navigate this new method and was 69% faster than the first observer who has more experience under cellulose treatment. where detection was more challenging because two larvae died from the second observer wheat bran treatment. Since one larva died from the first observer wheat bran treatment, this makes the detection times similar between observers under wheat bran treatment, with second observer being about 9% slower because of the two dead larvae.

Previous studies relied on conventional methods to locate implanted objects, which can be both time-consuming and dependent on observer experience, but our study demonstrates that ultraviolet light assist illumination can substantially reduce detection time, particularly under conditions where tissue contrast is bright. However, detection efficiency with

ultraviolet light is reduced in cases where larvae exhibit darkened tissues, which can obstruct filament visibility. Currently, direct comparisons of this study to other studies are limited due to the absence of prior ultraviolet-based approaches for monofilament detection in study organisms. The improvement observed is consistent with the principle that ultraviolet light enhances fluorescence contrast, thereby improving visual detection (Williams & Bridges, 1964). When exposed to ultraviolet light, objects embedded with ultraviolet reactive dye change from its original color to blue fluorescence. Such fluorescence can be measured using instruments such as fluorometers or spectrofluorometers, and determining the wavelengths associated with maximum fluorescence and excitation (Bowen & Wokes, 1953).

A potential limitation of this method is that it depends on how reactive the monofilament or object observed under the ultraviolet light is, which may not be effective if the material does not fluorescence or if fluorescence is weak, as well as how extremely dark the surrounding tissues are. The method is simple to implement and does not require specialized or expensive equipment beyond an ultraviolet light source. It also reduces the need for prolonged manipulation during dissection. This approach provides a faster, more practical, and accessible alternative for monofilament retrieval in experimental studies, while conventional methods often require prolonged searching and careful manipulation to identify the filament within the larval body, which can increase handling time.

The technique can be used easily by students who are new to the field, who find research fascinating and are interested in learning new things, as all that is needed is to flash the ultraviolet light beam onto the area from which they wish to retrieve the monofilament or object, provided it fluoresces. This accessibility supports active engagement in scientific investigation and inquiry (Hofstein and Lunetta, 1982) and enhances hands-on learning experiences that deepen understanding of experimental concepts (Clough, 2002), With the technique was developed to overcome limitations of conventional detection methods by improving visibility of objects that fluoresce, reducing detection time, and enabling more consistent detection and retrieval across different visibility qualities of the study organism.

5. Conclusion

Because the method is simple, cost effective, and easy to use, it has the potential to improve efficiency and reliability in experimental and laboratory-based research. The use of ultraviolet illumination provides a practical and effective approach for locating fluorescent implanted materials or small objects in study organisms, with possible applications extending beyond insect immunity studies, host–pathogen research, biomedical investigations, and other fields requiring recovery or visualization of fluorescent markers.

CRediT authorship contribution statement

Bridget Ugochi Anyanwueze: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Original draft, Review & editing.
Maria Ameh Ada: Investigation & Data curation
Paul Uchechukwu Ebo: Review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

References

Ayres JS, Schneider DS, (2009). The role of anorexia in resistance and tolerance to infections in Drosophila. PLoS Biology, Jul;7(7): e1000150.

Ardia, D.R., Gantz, J.E., B.C., Schneider, and Strebel, S. 2012, Costs of immunity in insects: an induced immune response increases metabolic rate and decreases antimicrobial activity. Functional Ecology, 26: 732-739. https://doi.org/10.1111/j.1365-2435.2012.01989.x

Bowen, E. J., and Wokes, F. (1953). Fluorescence of Solutions. Longmans, Green, London

Brennan CA, Anderson KV 2004. Drosophila: The genetics of innate immune recognition and response. Annu Rev Immunol 22: 457–483.

Clough, M.P. 2002. Using the laboratory to enhance student learning. In Learning Science and the Science of Learning, National Science Teachers Association, Editors: R. W. Bybee.

Calleri II DV, McGrail Reid E, Rosengaus RB, Vargo EL, Traniello JFA 2006. Inbreeding and disease resistance in a social insect: effect of heterozygosity on immunocompetence in the termite Zootermopsis angusticollis. Proc Roy Soc London B273: 2633–2640.

Downs CJ, Stewart KM 2014. Aprimer in ecoimmunology and immunology for wildlife research and management. *Calif Fish Game* 100:371–395.

Demas, G.E., Zysling, D.A., Beechler, B.R., Muehlenbein, M.P., French, S.S., 2011. Beyond phytohaemagglutinin: Assessing vertebrate immune function across ecological contexts. J. Anim. Ecol. 80, 710–730. https://doi.org/10.1111/j.1365-2656.2011.01813.x.

Dubovskiy, I.M., Kryukova, N.A., Glupov, V.V., Ratcliffe, N.A., 2016. Encapsulation and nodulation in insects. Inf. Syst. J. 13, 229–246.

Fellowes, and Godfray,2000. "The evolutionary ecology of resistance to parasitoids by Drosophila." *Heredity* 84.1: 1-8.

Freitak, Dalial, *et al*. 2003."Immune response is energetically costly in white cabbage butterfly pupae." *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270.suppl_2 : S220-S222.

Graham, A. L., Shuker, D. M., Pollitt, L. C., Auld, S. K. J. R., Wilson, A. J., & Little, T. J. (2011). Fitness consequences of immune responses: Strengthening the empirical framework for ecoimmunology. *Functional Ecology*, *25*(1), 5–17. https ://doi.org/10.1111/j.1365-2435.2010. 01777.x

Götz, P. 1986. Encapsulation in arthropods. Pages 153–170 in M. Brehélin, ed. Immunity in invertebrates. Springer, Berlin.

Gupta, A.P., 2019. Insect immunocytes and other hemocytes: roles in cellular and humoral immunity. In: Immunology of Insects and Other Arthropods. CRC Press, pp. 19–118.

Hofstein, A., and Lunetta, V.N. 1982. The role of the laboratory in science teaching: Neglected aspects of research. Review of Educational Research 52(2):201-217.

Hultmark, D. 1993. Immune reactions in Drosophila and other insects: a model for innate immunity. Trends Genet. 9:178–183

Johnsen, T. S., and M. Zuk. 1999. Parasites and trade-offs in the immune response of female red jungle fowl. Oikos 86:487–492.

Joyce EA, Kawale A, Censini S, Kim CC, Covacci A, *et al*. 2004. LuxS is required for persistent pneumococcal carriage and expression of virulence and biosynthesis genes. Infect Immun 72: 2964–2975.

Kraaijeveld AR, Godfray HCJ 1999. Geographic patterns in the evolution of resistance and virulence in *Drosophila* and its parasitoids. Am Nat 153(S5): S61–S74

Krams, I., Kivleniece, I., Kaasik, A., Krama, T., Freeberg, T.M., Rantala, M.J., (2013). Trade-off between cellular immunity and life span in mealworm beetles *Tenebrio molitor*. Current Zoology, 1 June; Pages 340–346.

Krams, I., Kecko, S., Kangassalo, K., Moore, F.R., Jankevics, E., Inashkina, I., Krama, T., Lietuvietis, V, Meija, L., Rantala, M.J., (2015). Effects of food quality on trade-offs among growth, immunity and survival in the greater wax moth *Galleria mellonella*: Food quality and life history trade-offs. Insect Science, Mar;22(3):431-9.

Kiss, Johanna, *et al*. 2020."Seasonal changes in immune response and reproductive investment in a biparental beetle." *Journal of insect physiology* 121: 104000.

Lowenberger, Carl A., *et al*. "Mosquito-PlasmodiumInteractions in Response to Immune Activation of the Vector." *Experimental parasitology* 91.1 (1999): 59-69.

Lochmiller RL, Deerenberg C.2000. Trade-offs in evolutionary immunology: Just what is the cost of immunity? Oikos.88:87–98.

Moreno-Garcia, M., Cordoba-Aguilar, A., Conde, R., Lanz-Mendoza, H., 2013. Current immunity markers in insect ecological immunology: assumed trade-offs and methodological issues. Bull. Entomol. Res. 103, 127–139. https://doi.org/10.1017/S000748531200048X

Ojala, K., Julkunen-Tiitto, R., Lindstr¨om, L., Mappes, J., 2005. Diet affects the immune defence and life-history traits of an Arctiid moth Parasemia plantaginis. Evol. Ecol. Res. 7, 1153–1170.

Ohmer, Michel EB, *et al*. 2021. "Applied ecoimmunology: using immunological tools to improve conservation efforts in a changing world." *Conservation Physiology* 9.1: coab074.

Pham, Linh N., *et al*. 2007."A specific primed immune response in Drosophila is dependent on phagocytes." *PLoS pathogens* 3.3: e26.

Qiu P, Pan PC, Govind S 1998.A role for the Drosophila Toll/Cactus pathway in larval hematopoiesis. Development 125: 1909–1920. 13.

Ryder, J.J., Siva-Jothy, M.T., 2001. Quantitative genetics of immune function and body size in the house cricket, *Acheta domesticus*. J. Evol. Biol. 14 (4), 646–653. https://doi.org/10.1046/j.1420-9101.2001.00302.x.

Rantala, M.J., Kortet, R., Kotiaho, J.S., Vainikka, A., Suhonen, J., 2003. Condition dependenceof pheromones and immune function in the grain beetle *Tenebrio molitor*. Funct. Ecol. 17, 534–540. https://doi.org/10.1046/j.1365-2435.2003.00764.x.

Rantala MJ, Roff DA 2006. Analysis of the importance ofgenotypic variation, metabolic rate, morphology, sex and development time on immune function in the cricket, Gryllus firmus. J Evol Biol 19: 834–843.

Rapkin, J., Jensen, K., Archer, C.R., House, C.M., Sakaluk, S.K., Castillo, E.D., Hunt, J., 2018. The geometry of nutrient space-based life-history trade-offs: sex-specific effects of macronutrient intake on the trade-off between encapsulation ability and reproductive effort in decorated crickets. Am. Nat. 191, 452–474. https://doi.org/ 10.1086/696147.

Stevens L, Yan G, Pray LA 1997. Consequences of inbreeding on invertebrate host susceptibility to parasitic infection. Evolution 51: 2032–2039.

Schmid-Hempel, P. 2003 Variation in immune defence as a question of evolutionary ecology. *Proc. R. Soc. Lond.* B 270, 357–366. (DOI 10.1098/rspb.2002.2265.)

Strand, M.R., 2008. The insect cellular immune response. Insect Science 15, 1–14. https://doi.org/10.1111/j.1744-7917.2008.00183.x.

Schmid-Hempel P. Evolutionary ecology of insect immune defenses. Annu Rev Entomol. 2005; 50:529-51. doi: 10.1146/annurev.ento.50.071803.130420. PMID: 15471530.

Schoenle LA, Downs CJ, Martin LB 2018. An introduction to ecoimmunology. In Cooper E, eds, *Advances in Comparative Immunology*.Springer, Cham, pp. 901–932.

Williams, R. Tecwyn, and J. Williams Bridges. 1964."Fluorescence of solutions: A review." *Journal of clinical pathology* 17.4: 371.

Williams, G. C. (1966). Natural selection, the costs of reproduction, and a refinement of Lack's principle. *American Naturalist*, *100*(916), 687– 690. https ://doi.org/10.1086/282461

Zettervall CJ, Anderl I, Williams MJ, Palmer R, Kurucz E, *et al*. 2004. A directed screen for genes involved in Drosophila blood cell activation. Proc Natl Acad Sci U S A 101: 14192–14197.