Foldscope - a versatile tool in agriculture for on-site and off-site identification of insects and pathogens

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Crops are vulnerable to a variety of biotic stresses during the agricultural production cycle. Few can be spotted visually while infested with insect pests and diseases, whereas others require laboratory diagnosis. In any event, early detection of pest incidence and proper identification of the culprit organism is critical for planning subsequent crop protection activities. Most disease causing pathogens and minute insects (aphids, whiteflies, thrips, mites, scale insects, mealy bugs, leafhoppers, plant hoppers, and other microscopic mites) make detection of the responsible organism difficult and impossible in many circumstances. Alternative identification instruments, such as an advanced lens or a microscope, are required in field conditions under such circumstances for identification and detection. However, in the laboratory, microscopic detection is a frequent practice for pest identification, but in field conditions, it is not practical since a compound microscope is heavy, requires power, and is difficult to transport to the field. As a result, there is a need for an alternate microscope that is easy to transport, small in weight, and economical.

With a philosophy of "microscopy for everyone," a team led by Manu Prakash and his student Jim Cybulski from Stanford University, USA, invented the origami-based optical microscope called foldscope that can be assembled from a flat sheet of paper and lens, as part of the "frugal science" movement that aims to make cheap and easy tools available for scientific use in the developing world. This foldscope's applications are unlimited, and it can be used for study and testing in a variety of industries. Foldscope can be attached to a smartphone using a magnet so that the user can photograph the magnification. When compared to traditional microscopes, it is compact and light (Cybulski et al., 2014). Foldscope microscopy has recently emerged as a useful imaging tool in a variety of sectors, including plant research, pollen studies, insect taxonomy and identification, disease detection, antioxidant testing, and in the medical field. In this article, emphasis has been given to its application in the field of agriculture for on-site and off-site identification of insects and pathogens.

Image acquisition using foldscope

Materials

Foldscope was procured online (Amazon, e-commerce site). The deluxe foldscope kit included paper foldscope sheet (140 x, 2 um resolution microscope) made up of a synthetic material which is waterproof, tear-resistant, and inexpensive, assembly/instruction sheet, lens, the sample-mounting components, a LED torch with a battery for illumination, cell-phone coupler to attach foldscope to smart phone, tardi sticker, unique ID sticker, clear circle sticker -1, double-stick ring sticker, diffuser stickers for light module, Reusable sealable PVC slides with micro-wells and plastic coverslips.

Assembling the foldscope

The foldscope kit is supplied with all the necessary parts and a user friendly assembly sheet with step-wise assemble. Also videos and detailed guidelines are available at https://foldscope.com/pages/user-guide._The total optical path length from the light source to the last lens surface is about 2.7 mm, while that of a conventional microscope is around 300 mm.

Imaging

The prepared slides were placed within the foldscope, and the slides were focused by sliding the foldscope's two scales. LED lights supplied with the kit were used as an external light source. Using magnetic couplers, a mobile phone (Redmi Note 6 Pro) was connected to the foldscope, and photographs were recorded after focusing using the smart phone. The photos acquired

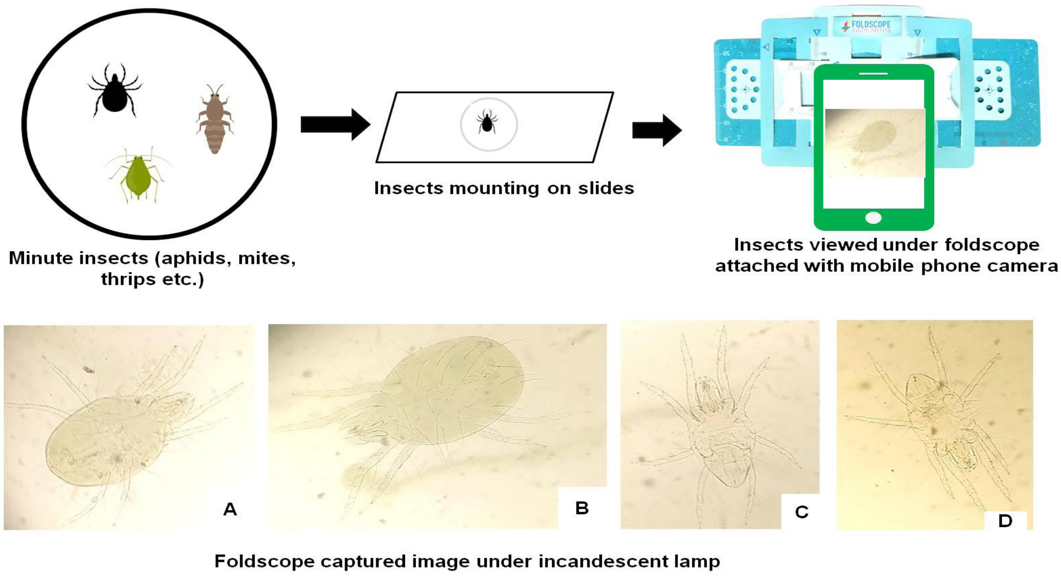

Fig. 1. Foldscope for insect identification: A&B - Female adults of *Tetranychus* sp. on marigold; C & D - Male adults of *Tetranychus* sp. on marigold

were utilized for identification.

Application of foldscope in on-site and off-site identification of insects and pathogens

Insects

Insects are the crucial components of many ecosystems, where they perform many important functions (Raghavendra et al., 2022). Insects are the major group on earth, making up 66% (1,020,007 species) of all animals and 82% of arthropods which includes few minute insects (aphids, whiteflies, thrips, mites, scale insects, mealybugs, leafhoppers, plant hoppers, and other microscopic insects) needs advanced lens or a microscope for identification and detection in the laboratory. Hence, there is a need for alternative tools for on-site and off-site identification of these insects with handy tools. The literature revealed that foldscope can be used as a handy tool for the identification of these minute insects (Figure 1). Prakya (2018) revealed that small pests of crop plants *viz.,* aphids, mites, thrips, and whiteflies and a few scale insects and crawlers of mealybugs can be easily observed under the Foldscope. He also stated that parasitoids (e.g., *Trichogramma chilonis*), predatory mites (e.g., *Amblyseius largoensis*, *Neoseiulus longispinosus*, *N. paspalivorus*) were easily visualized under the Foldscope.

Prakya (2020) conducted demonstration-cum-training workshops for traditional farmers in the states of Tripura and Meghalaya on the use of foldscope to observe microscopic arthropods like aphids, thrips, whiteflies, mites and, parasitoids, there was a remarkable turnaround in their perception of entomology. Through a series of workshops around 100 farmers were involved right from insect collection, sampling, staining, slide-mounting, and examining them through the Foldscope. Kulshreshtha et al., (2022) conducted field explorations of students to identify red spider mite (*Oligonychus coffeae* Nietner) infestations on tea leaves using foldcope and clear images of mites and the features of red spider mites were captured using foldscope. They also provided handson training to students on the visualization of mouthparts of arthropods with a foldscope and they could clearly visualize the ommatidia or compound eyes even with a foldscope.

Pathogens

Early diagnosis in the field is generally important to succeed in eradicating new diseases in modern agriculture to prevent massive production losses. Precision agriculture, using image processing and machine learning algorithms, has enormous benefits in detecting crop diseases at an early stage. There are numerous methods for identifying fungal phytopathogens; Pathogen culture on selective nutritional media and morphology assessment of the fungal colony using optical microscopy are two essential classical approaches for detecting and identifying fungi.

The key elements that affect the acceptability and choice of diagnostic tests are speed, specificity, sensitivity, and cost-effectiveness. Foldscope can be a very useful diagnostic instrument for the detection and identification of disease under field conditions. Wangadi et al. (2019) identified foliar fungal pathogens *viz., Cladosporium cladosporioides*, *Xylaria hypoxylon, Colletotrichum* sp., *Colletotrichum coffeanum, Rhizosphaera oudemansii*, *Alternaria alternata*, *Exobasidium vexans* associated with the leaf spot and leaf blight disease in the tea garden of Sikkim using Foldscope. Maheswari *et al.,* (2018) captured the images of *Alternaria solani,* early blight of tomato using foldscope, and the images were classified using various machine learning algorithms. The quadratic Support Vector Machine (SVM) classifier shows the highest classification accuracy of 89% in the prediction phase when compared to other machine learning algorithms

Chhetri et al. (2019) made an attempt towards on-site identification of plant pathogens infecting crops like rice, maize, ginger, beans, and some vegetables of Sikkim using foldscope. Foldscope was useful for on-site identification of the sclerotial body of sheath rot of rice, urediniospores of french bean, grass and canna rust, false smut of rice, powdery mildew conidia and conidiophore from vegetable crops, conidia of *Helminthosporium turcicum* from maize and *Cercospora beticola* from rayo saag. Additionally, the rhizosphere soils from the infected plants were also used to culture and identify the pathogens *viz.,* Conidia of *Fusarium* spp., and spores of rod shaped bacteria.

Singh et al. (2018) used the foldscope for *in situ* identification of the soft rot complex of ginger and revealed that transverse sections of healthy rhizome showed outer and inner cork, parenchymatous ground tissue, endodermis, a vascular bundle containing xylem, phloem, fibers, and oleoresin cells arranged in a uniform fashion, whereas the section of diseased rhizome showed disintegrated parenchymatous tissue, endodermis, a vascular bundle, and oleoresin. Arora et al. (2020) monitored superoxide production and cell death during pathogen infection in *Arabidopsis* under different nitrogen regimes. Prakya (2018) studied the importance of foldscope in biocontrol research and revealed that foldscope can be used to differentiate between fungal bio-control agents (e.g., plant disease antagonists - *Trichoderma* spp.; entomopathogenic fungi - *Beauveria bassiana*, *Metarhizium anisopliae*, *etc*.) and plant pathogens and nematodes which are easily visualized under the Foldscope.

Conclusion

Foldscope offers a handy and cost-effective tool for on-site and off-site identification of insects and pathogens by the farmers which in turn, facilitates the timely planning and execution of insect pests and diseases management tactics.

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