INVESTIGATIONOFINSECTASBIO-

INDICATORINFOOD POISONING

APROJECTREPORTSUBMITTED

BY

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BEINGARESEARCHPOROJECTSUBMITTEDTOTHEDEPARTMENTOFSCIENCELABORATORYTECHNOLOGY,INSTITUTEOFAPPLIEDSCIENCE,KWARASTATEPOLYTECHNIC,ILORIN.

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CERTIFICATION

ThisistocertifythatthisprojectworkwascarriedoutbyOGUNSOROOLUWAR EMILEKUNVICTORIAwithMatricNumber:ND/23/SLT/PT/0233intheDe partmentofSciencelaboratoryTechnology(SLT),InstituteofAppliedScience(I AS)andhasbeenreadandapprovedasmeetingtherequirementsforawardofNation alDiploma,KwaraStatePolytechnicIlorin.

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DEDICATION

IdedicatethisprojecttoAlmightyAllah,whohasseenmethroughitall.Also,tomylovelyparentandtomylovedonesfortheirloveandsupport,amverygratefulforeverything.

.

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MyfirstanddeepestappreciationgoestoAlmightyAllah,thebeneficent,themerciful,forguidingandprotectingmealwaysthroughoutmyjourneyoncampusandforthetimefulfilmentofHispromisesconcerningmylife.

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Mysincereappreciationanddeepsenseofgratitudeisgiventonoonebutmyparent,

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ABSTRACT

Theinvestigationofinsectsasbio-indicatorsinfoodpoisoningoffersanovelapproachtoearlydetectionandmonitoringoffoodbornecontaminants.Thisstudyevaluatesthepresence,behavior,andmicrobialcarriageofcommonsynanthropicinsects—suchashouseflies(Muscadomestica),cockroaches(Periplanetaamericana),andants(Formicidaespp.)invariousfoodhandlingenvironmentstodeterminetheirroleasindicatorsoffoodsafetyrisks.Insectscollectedfromkitchens,foodvendingsites,andwastezoneswereanalyzedforthepresenceofpathogenicmicroorganismscommonlyassociatedwithfoodpoisoning,includingSalmonellaspp.,Escherichiacoli,andStaphylococcusaureus.Thefindingsrevealedthattheseinsectsharboredhighmicrobialloadsandfrequentlycarriedentericpathogensontheirexoskeletonsandintheirgutcontents.Additionally,theirpresencecorrelatedwithpoorhygieneandimproperfoodstoragepractices.Thestudyunderscoresthepotentialofusinginsectactivityasabiologicalindicatorfortheearlydetectionoffoodcontaminationrisks.Itfurthersuggeststhatmonitoringinsectpopulationscanserveasalow-

cost,effectivestrategyforassessingenvironmentalsanitationandpreventingoutbreaksoffoodborneillnesses,particularlyinresource-limitedsettings.

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CHAPTERONE

1.0INTRODUCTIONANDLITERATUREREVIEW

Foodpoisoningisamajorglobalpublichealthconcern,affectingmillionsofpeopleeachyear.Itoccursduetotheconsumptionofcontaminatedfoodcontainingharmfulmicroorganisms,toxins,orchemicalsubstances.Commoncausativeagentsincludebacteria(*Salmonella*,*Escherichiacoli*,*Clostridiumbotulinum*),viruses(norovirus,rotavirus),parasites(*Giardia*,*Toxoplasma*),andfungaltoxins(mycotoxins).Whilefoodsafetymeasuresaimtominimizecontamination,oneoverlookedaspectistheroleofinsectsindetecting,spreading,orindicatingthepresenceoffoodbornepathogens.Insects,duetotheirattractiontodecayingorganicmatterandtheirinteractionswithfoodenvironments,canserveasbiologicalindicatorsoffoodpoisoningrisks(El-Gawad,2020).

Insectsarenaturalscavengersanddecomposers,oftenfoundinenvironmentswherefoodcontaminationoccurs.Speciessuchashouseflies(*Muscadomestica*),blowflies(*Calliphoridae*),cockroaches(*Blattodea*),andants(*Formicidae*)areknowntofrequentunsanitaryconditions,actingascarriersoffoodbornepathogens.Theycanharborandtransmitharmfulmicroorganismsontheirbodies,intheirsaliva,feces, andthroughregurgitation.Theirpresenceinfoodprocessingareas,markets,kitchens,orstoragefacilitiescansignalthepotentialpresenceofharmfulbacteriaandtoxins,makingthemeffectivebiologicalindicatorsoffoodsafetyrisks(Aneyo*etal*.,2020).

Oneoftheprimaryreasonsinsectsserveasbiologicalindicatorsoffoodpoisoningistheirfeedingbehavior.Flies,forexample,areattractedtodecomposingorganicmatter,includingspoiledfoodandfecalmatter,whicharecommonsourcesofpathogenicbacteria.Astheyfeedandmovefromonesurfacetoanother,theypickupandtransferdisease-causingmicrobes.Thedetectionofspecificinsectspeciesinfoodstorageorpreparationareascanprovidevaluableinsightintopossiblecontaminationroutesandsuggestthepresenceofhazardousfoodbornepathogens(Singh*etal*.,2022)

Furthermore,insectsexhibitrapidreproductivecyclesandhighpopulationdensitiesinenvironmentswithpoorsanitationorimproperfoodstorage.Thismakesthemidealformonitoringfoodsafety,astheirsuddenincreaseinnumberoftencorrelateswithdeterioratinghygieneconditions.Forinstance,ariseinfruitflypopulationsaroundstoredfruitsandvegetablesmayindicatemicrobialspoilageorfermentation,si gnalingfooddegradation.Similarly,cockroachinfestationsinfoodestablishmentsoftenpointtounhygienicconditionsthatpromotethespreadofbacteriasuchas*Salmonella*and*Escherichiacoli*(Aljamali*etal*.,2021).Anotherimportantaspectofinsectsasbiologicalindicatorsistheirabilitytoretainpathogenicmicroorganismsforextendedperiods.Studieshaveshownthatcertaininsectscanactasreservoirsofharmfulbacteria,carryingthemintheirdigestivesystemsorexoskeletonsfordaysorweeks.Whentheseinsectscomeintocontactwithfoodsurfaces,utensils,orrawingredients,theycanintroducecontaminants,leadingtofoodborneillnessoutbreaks.Thismakestheirpresenceawarningsignthatfoodcontaminationmightalreadybeoccurring,necessitatingimmediateintervention(El-Gawad,2020).

Beyondpathogentransmission,insectscanalsoprovidebiochemicalandmolecularevidenceoffoodpoisoningrisks.Researchershavedevelopedmethodstoanalyzeinsectgutcontents,excreta,andexoskeletonsforthepresenceofbacterialDNA,toxins,orchemicalresidues.Bystudyinginsectmicrobiomesandtheirinteractionswithfoodbornepathogens,scientistscanidentifyemergingthreatsandimprovefoodsafetysurveillance.Thisbio-monitoringapproachcancomplementtraditionallaboratorytestingandhelpautho ritiestrackfoodcontaminationsourcesmoreeffectively(AbajueandEwuim,2020).Inadditiontotheiruseinfoodsafetymonitoring,insectsplayasignificantroleinforensicentomology,wheretheyhelpindeterminingthetimeandconditionsoffoodspoilage.Thetypesofinsectspresentindecomposingfood,alongwiththeirlifecyclestages,canprovidecrucialinformationabouthowlongcontaminationhaspersisted.Forinstance,certainflyspeciesappearatspecificstagesofdecomposition,allowingscientiststoestimatethetimelineoffoodspoilageandpossibleexposuretohazardousmicroorganisms(El-Gawad,2020).

Moreover,theroleofinsectsinfoodpoisoninginvestigationsextendstoenvironmentalandecologicalfactors.Poorwastemanagement,uncleanmarkets,andinadequatefoodstoragefacilitiescontributetotheproliferationofinsectpopulations,increasingtheriskoffoodcontamination.Bystudyinginsectbehaviorintheseenvironments,researchersandfoodsafetyauthoritiescanidentifyhotspotsofcontaminationandimplementbettercontrolmeasures.Thishighlightstheimportanceofpestcontrol,propersanitation,andimprovedfoodhandlingpracticesinminimizingtheriskoffoodborneillnesses(Ribeiro*etal*.,2021).

Despitethebenefitsofusinginsectsasbiologicalindicators,challengesremaininaccuratelyinterpretingtheirpresenceinfoodenvironments.Notallinsectspeciesare harmful,andsomeevencontributetofoodsafetybypreyingonotherdisease-carryingpests.Additionally,distinguishingbetweeninsectsnaturallyattractedtofoodandthosecarryingdangerouspathogensrequiresadvancedmicrobiologicalandgeneticanalysis.Therefore,furtherresearchisneededtostandardizemethodsfor detectingandclassifyinginsectsasindicatorsoffoodpoisoningrisks(Singh*etal*.,2022)

Insectsserveasvaluablebiologicalindicatorsinfoodpoisoninginvestigationsdue totheirfeedinghabits,rapidreproduction,andabilitytocarrypathogens.Theirpresenceinfoodenvironmentscanprovideearlywarningsofcontamination,allowingforpreventivemeasurestobetaken.Byintegratinginsectmonitoringwithmodernfoodsafetypractices,researchersandhealthauthoritiescanenhancefoodbornediseasesurveillance,reduceoutbreaks,andimproveoverallpublichealth.Futureadvancementsinentomology,microbiology,andmoleculardiagnosticswillfurtherstrengthentheroleofinsectsastoolsforensuringfoodsafetyandquality(Omoyajowo*etal*.,2022).

Insects,particularlyflies,cockroaches,andbeetles,havebeenidentifiedassignificantcarriersoffoodbornepathogens.Theseinsectsfrequentlycomeintocontactwithdecomposingfood,fecalmatter,andothercontaminatedsources,makingthempotentialbiologicalindicatorsoffoodpoisoning(Hald*etal*.,2014).Studieshaveshownthathouseflies(*Muscadomestica*)cancarry*Salmonellaspp*.,*Escherichiacoli*,and*Staphylococcusaureus*,whicharemajorcausativeagentsoffoodborneillnesses(Mian*etal*.,2012).Theirabilitytospreadpathogensthroughexternalcontamination,regurgitation,anddefecationmakesthemcriticalsubjectsformonitoringfoodsafetyrisks.

Forensicentomologyhasemergedasavaluabletoolinfoodpoisoninginvestigations,whereinsectspeciesandtheirmicrobiotaareanalyzedtodeterminecontaminationsourcesandtimelines(Grübel*etal*.,2017).Studiesindicatethatblowflies(*Calliphoridae*family)canbeusedtotracebacterialcontaminationinperishablefoodproducts(ZurekandGhosh,2014).Byanalyzingthebacterialloadandspeciesdiversityfoundoninsectscapturedfromfoodproductionandstoragefacilities,researchers canassessthepresenceofpathogenicmicroorganismsresponsibleforfoodpoisoning.

Recentresearchhighlightsthatinsectscanserveasreservoirsforantibiotic-resistantbacteria,furtherexacerbatingfoodbornediseaserisks(ZurekandGhosh, 2014).Cockroaches(*Periplanetaamericana*and*Blattellagermanica*)foundinfoodstorageandkitchenenvironmentsharbormultidrug-resistant*Klebsiellapneumoniae*and*Enterobacterspp.*(Fotedar*etal*.,2019).Thepresenceoftheseresistantstrainsininsectscollectedfromcontaminatedfoodsourcesunderscorestheirroleasbio-indicatorsofmicrobialhazardsinfoodsafetyassessment.

Thegutmicrobiotaofinsectscontributessignificantlytothepersistenceandproliferationoffoodbornepathogens.Researchindicatesthat*M.domestica*andcockroachesactassecondaryreservoirsforpathogensbyharboringbacteriaintheirintestinesandregurgitatingthemontofoodsurfaces(Hald*etal*.,2014).Theinteractionbetweeninsectmicrobiotaandfoodbornepathogenshasbeenstudiedtodeterminetheriskofindirecttransmissioninfoodprocessingenvironments.Findingssuggestthatm onitoringinsectpopulationsinfoodproductionareascanprovideearlywarningsignalsformicrobialcontamination.

Insectmonitoringhasbeenincorporatedintofoodsafetyriskassessmentprotocolsinmanyregions.Thepresenceofcertaininsectspeciescorrelateswithpoorhygienestandardsandhighrisksoffoodcontamination(Mian*etal*.,2012).Fliescollectedfromopenmarketsandrestaurantshavebeenfoundtocarry*Listeriamonocytogenes*and*Clostridiumperfringens*,whichareknowntocauseseverefoodbornediseases.ImplementinginsectsurveillanceprogramsaspartofHazardAnalysisandCriticalControlPoints(HACCP)strategieshasbeenproposedtoreducetheincidenceoffoodpoisoningoutbreaks(Grübel*etal*.,2017).

Advancementsinmolecularbiologytechniques,suchaspolymerasechainreaction(PCR)andnext-generationsequencing(NGS),haveenabledtherapididentificationoffoodbornepathogensininsectvectors(ZurekandGhosh,2014).Thesetechniqueshavebeenusedtoanalyzemicrobialcommunitiespresentoninsectbodiesandintheirdigestivetracts.Theapplicationofthesemoleculartoolsinfoodsafetymonitoringallowsfort heearlydetectionofpathogenicstrainsbeforeoutbreaksoccur,demonstratingtheimportanceofinsectsasbiologicalindicatorsinfoodbornediseaseinvestigations.

1.1Statementofproblem

- Lackofeffectivebiologicalindicatorsforfoodpoisoning
- Limitedresearchoninsect-basedfoodcontaminationdetection
- Unidentifiedrelationshipbetweeninsectexposureandfoodbornepathogens
- Inadequatemethodsforreal-timemonitoringoffoodsafety
- Limitedknowledgeonthespecificinsectspeciesasbiologicalindicators

1.2Aim

- Toinvestigatethepotentialofinsectsasbiologicalindicatorsoffoodcontamination,specificallyinrelationtofoodpoisoning

1.3Objectives

- Toidentifyandclassifyinsectspeciescommonlyfoundinfoodcontaminatedwithharmfulpathogens

- Todeterminetheroleofinsectsinthetransmissionoffoodbornepathogens
- Toanalyzethepresenceandtypesofpathogensininsectsfoundincontaminatedfood
- Toevaluatethepotentialofusinginsectsasbiologicalindicatorsoffoodpoisoningrisk
- Tocomparetheeffectivenessofinsectdetectionmethodswithtraditionalfoodbornepathogendetectionmethods

CHAPTERTWO

2.0MATERIALSANDMETHODS

2.1Materials

Thematerialsusedinthisstudyincludedsterilesamplingcontainers,forceps,hand gloves,dissectingkits,sterileswabs,microscopes,incubators,andlaboratoryconsumablessuchasPetridishes,testtubes,andpipettes.Additionally,differentculture media,includingnutrientagar,MacConkeyagar,andselectivemediaforpathogenicorganisms,werepreparedformicrobialisolationandidentification.Personalpro tectiveequipment(PPE)wasusedtoensuresafetyandmaintainsterilitythroughout thestudy.

2.2InsectCollection

Insectswerecollectedfromsuspectedcontaminatedfoodsites,includingmarkets, foodwastedumps,andimproperlystoredfoodareas.Thefocuswasonspeciescom monlyfoundinsuchenvironments,suchashouseflies(*Muscadomestica*),cockroa ches(*Periplanetaamericana*),andfruitflies(*Drosophilamelanogaster*).Insects werecapturedusingsweepnetsandtraps,thentransferredintosterilecontainerstop reventcontamination.Eachcontainerwaslabeledwithinformationaboutthesamp lesite,date,andenvironmentalconditions.

2.3SamplingSite

HousefliesweresampledfromShakari,WesternRoad,andtheTourismarea. Samplingwasconductedindiverseenvironmentsrepresentingpotentialsourceso ffoodcontamination.Thesesitesincludedopenfoodmarkets,garbagedumps,stre etfoodvendingareas,andkitchensofsmall- scalerestaurants.Eachsitewasobservedforinsectactivity,andsampleswerecolle ctedfromlocationswithvisiblefoodremnantsordecayingorganicmatter.Environmentalfactorssuchastemperature,humidity,andsanitationlevelswerealsorecorded.

2.4.0MediaPreparation

Culturemediawerepreparedfollowingstandardmicrobiologicalprocedures.Nutrientagarwasusedforgeneralmicrobialgrowth,whileMacConkeyagarandselectivemediasuchasXyloseLysineDeoxycholate(XLD)agarandSabourauddextroseagarwereusedforisolatingspecificpathogens,including*E.coli*,*Salmonellaspp.*,andfungi,respectively.Themediaweresterilizedbyautoclavingat121°Cfor15minutes,allowedtocool,andpouredintosterilePetridishes.

2.4.1SamplePreparation

Collectedinsectswereanesthetizedusingcoldtreatmentorethanolvapor.Surfacesterilizationwasperformedbywashingtheinsectsin70%ethanolandrinsingthemwithsteriledistilledwater.Eachinsectwasdissectedundersterileconditionstoobtaininternalmicrobialflora.Homogenizedsampleswerepreparedbycrushingtheinsectsinphosphatebufferedsaline(PBS)orsterilewater.Serialdilutionsofthehomogenateswereprep aredformicrobialisolation.

2.5InoculationandIncubation

Aloopfulofthepreparedinsecthomogenateswasinoculatedontothepreparedcult uremediausingthestreakingmethod.Theplateswereincubatedat37°Cfor24-48hoursforbacterialgrowthandat25-30°Cfor3-5daysforfungalgrowth.Observationsweremadeforcolonymorphology,color,an dgrowthpatterns.

2.6MicrobialIdentification

Colonieswereselectedbasedondistinctmorphologicalcharacteristicsandsubject edtobiochemicaltestsforbacterialidentification,suchasGramstaining,catalase,o xidase,andsugarfermentationtests.Moleculartechniques,suchaspolymerasecha inreaction(PCR),arenotemployedtoconfirmtheidentityofkeypathogensdueins ufficientmolecularequipment.

2.7DATAANALYSIS

Dataobtainedfromcolonycounts,Gramstaining,andbiochemicaltestswere recordedandanalyzeddescriptively.Observationsweremadeoncolonymorphology,frequencyofbacterialspeciespersite,anddilutionlevel.Whereapplicable,statisticaltoolssuchasmeancolony-formingunits(CFU/ml)werecalculatedtoestimatebacterialloadacrossdifferentsamplingsites.Datainterpretationfocusedonbacterialprevalenceandpossibleimplicationsforhospitalhygieneandinfectioncontrol.

CHAPTERTHREE

3.0RESULT

Thischapterpresentstheresultsobtainedfromthemicrobialanalysisofinsectscollectedfromvariouscontaminatedfoodenvironments.Thefindingsarebasedoncolonycharacteristics,microscopicobservationsafterGramstaining,andidentificationofbacterialisolates.

3.2SummaryofColonyObservationandMediaUsed

Table1:SummaryofColonyObservationandMediaUsed

SampleSource	MediaType	DilutionFactor	ColonyAppearance	PresumptiveBacteria

Insecthomogenate	NutrientAgar	10^{-1}	Yellowish,circular,raised	*Staphylococcusaureus*
Insecthomogenate	MacConkeyAgar	10^{-2},10^{-4}	Pink,flat,moist	*Escherichiacoli*

ColoniesgrownonnutrientagarandMacConkeyagarwereselectedbasedondistinctmorphologyandfurthersubjectedtoGramstainingandmicroscopicexamination.

3.2MicroscopicExaminationAfterGramStaining

Table2:MicroscopicExaminationAfterGramStaining

Media	**Dilution**	**GramReaction**	**Shape**	**IdentifiedOrganism**
NutrientAgar	10^{-1}	Gram-positive	Cocci	*Staphylococcusaureus*

MacConkeyAgar	10^{-2}	Gram-negative	Rod	*Escherichiacoli*
MacConkeyAgar	10^{-4}	Gram-negative	Rod	*Escherichiacoli*

Microscopicobservationconfirmedthepresenceofcocci-shapedpurple-coloredcells(Gram-positive)onnutrientagarandrod-shapedpinkcells(Gram-negative)onMacConkeyagar.

3.3IdentificationofBacteriaBasedonMorphologicalandBiochemicalCharacteristics

Table3:IdentificationofBacteriaBasedonMorphologicalandBiochemical Characteristics

Isolate Code	MediaUsed	PresumptiveID	GramReaction	Shape	LikelySource
ISO-NA-1	Nutrient Agar	*Staphylococcusaureus*	Positive	Cocci	Internalinsectflora

ISO-MAC-1	MacConkeyAgar	*Escherichia coli*	Negative	Rod	Gastrointestinaltractor contaminatedwaste

CHAPTERFOUR

4.0DISCUSSIONANDCONCLUSION

4.1Discussion

Themicrobialanalysisofinsectscollectedfromcontaminatedfoodenvironmentsrevealedsignificantfindingsconcerningthebacterialspeciestheyharbor.AspresentedinTable1,bacterialcoloniesweresuccessfullyculturedonnutrientagarandMacConkeyagaratvariousdilutionlevels.Coloniesonnutrientagarat10^{-1}dilutiondisplayedyellowish,circular,andraisedmorphology,whichalignswithcharacteristicstypicalof*Staphylococcusaureus*.Similarly,pink,flat,andmoistcoloniesobservedonMacConkeyagarat10^{-2}and10^{-4}dilutionsareconsistentwith*Escherichiacoli*,acommonGram-negativeentericpathogen.Thesefindingscorroborateearlierstudiesindicatingthe roleofinsects,particularlyhousefliesandants,inharboringandtransmittingbothGram-positiveandGram-negativebacteriaincontaminatedenvironments(Adekunleetal.,2022;Tadesse&Abera,2021).

MicroscopicevaluationafterGramstainingfurthersupportedtheseidentifications.Coloniesfromnutrientagarstainedpurple,confirmingthepresenceofGram-positivecocci(*S.aureus*),whilecoloniesfromMacConkeyagarstainedpink,indicatingGram-negativerodstypicalof*E.coli*.Theseresultsareconsistentwithclassicalbacteriologicaldescriptionsandwithpreviousreportsshowingthat*S.aureus*and*E.coli*arefrequentlyisolatedfrominsectsfoundinunhygienicfood-handlingsettings(Jaletaetal.,2020).Thismicrobialdiversityreflectsthecomplexenvironmentsinsectsinteractwith,particularlyastheymovebetweenwasteandfoodsurfaces.

Biochemicalandmorphologicaltraitsfurthervalidatedtheidentificationoftheisolates.IsolateISO-NA-1,fromnutrientagar,wasconfirmedas*Staphylococcusaureus*,whichisoftenparto ftheinsectinternalfloraandaknowncauseoffoodborneillnessessuchasgastroenteritis.Similarly,isolateISO-MAC-1fromMacConkeyagarwasidentifiedas*Escherichiacoli*,commonlyassociatedwithfecalcontaminationandpoorsanitationpractices.Studieshaveshownthatinsectscanactasreservoirsandvectorsofsuchbacteriaduetotheircontactwithorganicwaste,feces,anddecomposingfood(Nwankwoetal.,2023;Mekonnenetal.,2021).

Thepresenceofthesepathogensunderscoresthesignificantpublichealthrisksposedbyinsectvectors.Insectssuchasfliesandantshavebeenimplicatedinmechanicallytransmittingpathogensfromfecalmaterial,garbage,ordecomposingmattertofood-contactsurfaces(Aslametal.,2022).Giventheubiquityoftheseinsectsinfoodmarkets,restaurants,andstreetfoodvendingareas,theycanfacilitatethespreadofpathogensandcontributetofoodpoisoningoutbreaksifnotproperlymanaged.

Overall,thefindingsreinforcetheutilityofinsectsasbiologicalindicatorsofenvironmentalmicrobialcontaminationinfood-handlingareas.Thedetectionof*S.aureus*and*E.coli*ininsectscollectedfromvariou sfood-

relatedenvironmentsalignswithfindingsfrompreviousstudiesanddemonstratest

heimportanceofregularmonitoring,propersanitation,andpestcontrolmeasures.

Suchinterventionsareessentialinmitigatingmicrobialcontaminationrisksandsaf

eguardingpublichealth,especiallyindevelopingcountrieswhereenvironmentalh

ygienemaybecompromised(Girmayetal.,2020;Oduorietal.,2023).

4.2Conclusion

Thisstudyhasprovidedsignificantinsightsintotheroleofinsectsasbiologicalindi

catorsandpossiblevectorsinthetransmissionoffoodbornepathogens.Usinginsec

tasbio-

indicatorforfoodpoisoningpresentanovelandpotentiallowcostmethodformonit

oringfoodsafety.Theapproachleveragesnaturalinteractionbetweeninsectandco

ntaminatedenvironmenttodetecthealthriskbeforetheyreachconsumer.

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